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ERRATA AND AUTHORS' EMENDATIONS

- Page 1, lines 25 and 29, page 14, lines 1 and 6, and page 28, eighth line from bottom, "U. S. 875" should be "U. S. 785."
- Page 50, fourth line from bottom, "and" should be "of."
- Page 96, fourth line from bottom, "(4r)" should be "(14r)."
- Page 98, line 3, "(4.5)" should be inserted after "temperature."
- Page 118, Table 10, experiments 11, 12, in column 11 headed "Maximum," footnote reference "(a)" should be "(b)"; experiment 8, in column 11, "3" should be "34," and in column 12 headed "Index for optimum temperature" "35" should be "3."
- Page 128, after literature citation (9), "WITT, A. W." should be inserted after "KNIGHT, R. C."
- Page 131, line 33, insert between "*giganteum*" and "in" the following, "on creeping bent in putting greens of golf courses."
- Page 263, line 19, "Raos" should be "Rosa."
- Pages 358-359, Table 1, and pages 360-361, Table 2, last column headed "Period of survival," "Grams" should be "Days."
- Page 500, third line from bottom, insert "(Fig. 2, C.)" after "crystals."
- Page 518, twelfth line from bottom, omit "C."
- Page 552, Table 2, third line from bottom, "Protein ($\times 6.25$)" should be "Protein ($N \times 6.25$)."
- Page 554, Table 4, omit superscript "a" after "Ratio of" and its corresponding footnote.
- Page 610, line 4, reference to Reimer should be (23), not (22, 23).
- Page 613, line 9, and page 614, line 13, reference to Reimer should be (22), not (22, 23).
- Page 681, twenty-sixth line from bottom, under column headed "Plants erect at harvest," "84.1" should be "81.4."
- Page 724, fourth line from bottom, "species" should be "specimens."
- Page 739, line 4, (10) should be (9); line 17, (10) should be (9) and (9) should be (10).
- Page 748, Figure 1 should be inverted.
- Page 761, nineteenth line from bottom, "two" should be "three."
- Page 796, Table 1, fourth column, under "Moyock" the "Do." opposite "Hahto" should be opposite "I!aberlandt."
- Page 799, Table 3, eighth column, under "Average coefficient" opposite "Columbia," "0.9" should be "0.09."
- Page 896, line 15, "1,000" should be "100."
- Page 963, eighth line from bottom, "gm." should be "c. c."

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No. 1

DEVELOPMENT OF FLOWER AND SEED OF SOME VARIETIES OF SUGAR CANE¹

By ERNST ARTSCHWAGER, *Associate Pathologist*, E. W. BRANDES, *Principal Pathologist in Charge*, and RUTH COLVIN STARRETT, *Assistant Cytologist*, Office of Sugar Plants, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The investigation presented in this paper is part of a comprehensive study of the anatomy, the ontogeny, and the cytology of sugar cane, undertaken primarily as a basis for further research in diverse fields including taxonomy, physiology, pathology, and genetics. The Office of Sugar Plants now has projects in all these fields, and it is proposed by the present series of studies to lay a foundation that will be useful in advancing them. A previous publication which discussed the anatomy of the vegetative organs of sugar cane (1)² was based on a critical examination of a single variety, Louisiana Purple (Zwart Cheribon), of the species *Saccharum officinarum*, to which belongs the large group of thick-stemmed or "noble" cane varieties, preferred, where they can be successfully grown, by all commercial cane planters. It is recognized that owing to the diverse forms of *Saccharum* it is unsafe to generalize on the basis of a study of one variety or a limited number of varieties. Therefore the logical plan is to study a single variety in detail, and to follow this with comparative studies of the minute anatomy of selected parts, which will doubtless reveal differences that may be useful in helping to separate and identify varieties. Studies of the inflorescence are especially difficult and narrowly circumscribed, in so far as the range of selection of varieties is concerned, on account of irregularity in flowering of sugar cane, which takes place only when environmental conditions are exceptionally favorable and, moreover, on account of the frequently encountered degeneracy or abortive development of the male and female organs. The varieties used in the present study, U. S. 875 and U. S. 1694,³ were those available in sufficient quantity in the United States sugar-plant field experiments at Canal Point, Fla., when the investigation was begun in January, 1927. The variety U. S. 875 is from the F₂ progeny of a cross between *Saccharum officinarum* (Zwart Cheribon variety) and *S. spontaneum*, a wild species; and U. S. 1694 is from the F₂ progeny of a cross between *S. officinarum* (Zwart Cheribon variety) and *S. barberi* (Chunnee variety).

According to Jeswiet (12, 13), the genus *Saccharum* embraces four species: *S. spontaneum*, *S. sinense*, *S. barberi*, and *S. officinarum*.

¹ Received for publication Aug. 10, 1928; issued July, 1929. This paper reports the second of a series of investigations of the anatomy of sugar cane. The first was entitled "Anatomy of the Vegetative Organs of Sugar Cane" (1).

² Reference is made by number (italic) to "Literature cited," p. 29.

³ U. S. = United States Department of Agriculture seedling.

Another species, provisionally named *S. robustum*,⁴ was found in New Guinea by Doctor Jeswiet when he accompanied the United States Department of Agriculture airplane expedition to the interior of that island in 1928. In the following key, which has been translated from Jeswiet's original paper, are given the characters on which the separation of the species is founded:

KEY TO THE SPECIES OF SACCHARUM

- A. Main axis of inflorescence and cluster axes with long hairs. Glumes always 4. Lodicules either ciliate or not. If the spikelets of the same pair do not flower simultaneously, the pedicellate one always blooms first. Culms green, grayish green, greenish bronze, ivory, or white.
 1. Lodicules ciliate, long subterranean runners present, growing wild ----- *Saccharum spontaneum*.
 2. Lodicules not ciliate. Subterranean runners short. Sugar-producing, cultivated plants.
 - a. Leaves broad (to 50 mm.). Long cane species, nodes all fusiform, greenish bronze (among others, Uba cane) ----- *Saccharum sinense* Roxb. amend. Jeswiet.
 - b. Leaves narrow, short inconspicuous cane species. Nodes usually cylindrical, grayish green, white, or ivory. Rather limited to British India (among others, Chunnee cane) ----- *S. barberi* Jeswiet.
- B. Main axis of the inflorescence never having long hairs, often glabrate; rachis nodes glabrate or with very few hairs. Glumes generally 3, sometimes 4. Lodicules not ciliate. If the spikelets of the same pair bloom at different times, the sessile one always is the first. Culms differing in color from pale or dark green to dark yellow, dark red, violet, often striped. Cultivated plant ----- *Saccharum officinarum*.
 1. Fourth glume present. Vigorous plants with low sugar percentage. Types: Among others, Fidji, Ardjoeno, Green Coerman (Corven Duitsch), New Guinea.
 2. Fourth glume wanting. Plants with a general high percentage of sugar. Types: Among others, Cheribon, Batjan, Borneo, Bandjermasia, Preanger.

It will be noted that the separation of species in this key is based on the panicle characters. In seeking to establish a basis for varietal determination it is necessary to make use of the vegetative characters as well. Barber (2) in his monumental work on the sugar canes of India considered the characters of stem, leaves, and roots. Various investigators have made use of special characters such as the number and arrangement of the stomata, the number and disposition of root primordia in the root ring, and the character and arrangement of emergences on the buds and leaves; but no system has been found sufficiently practicable to commend itself to general use, and it has become increasingly apparent that the problem is so complicated that accurate determination of cane varieties is a matter for specialists. In the present investigations anatomical characters have been observed to vary sufficiently in the different varieties to be of value in supplementing the morphological characters ordinarily used in attempting to separate varieties.

METHODS

The flower material was fixed in Carnoy's fluid directly in the field. The material was embedded without removing the scales, since it was found that in young material the scales are not sufficiently hard to interfere with the cutting, and in older material the presence of the scales, by preventing too rapid dehydration and infiltration with paraffin, resulted in better preservation of the nuclei. Mature seed

⁴ The new species *Saccharum robustum* is not distinguishable from *S. officinarum* on the basis of panicle characters thus far studied.

fixed while still in the milk stage could be cut comparatively easily even if embedded in paraffin. Older seed had to be embedded in celloidin. All cytological material was stained with Haidenhein's iron-alum hematoxylin, and histological material with Delafield's hematoxylin, and counterstained with saffranin.

All photomicrographs were taken on Wratten M plates with B-58 and E-22 filters used singly and in combination. The drawings⁵ are based on photomicrographs or their enlargements.

THE INFLORESCENCE

The inflorescence of sugar cane forms an open panicle. (Fig. 1, A.) Every species and almost every variety has, according to Jeswiet (13), its peculiar type of inflorescence or "arrow." Depending on the type of lateral axes, the arrow will be small or large, broad or narrow, conical or cylindrical. The main axis of the inflorescence is longest in *Saccharum officinarum*, shortest in *S. spontaneum*, and intermediate in *S. sinense*. It is thick, straight, and round at the base, becoming gradually thinner, sinuous, and distinctly grooved higher up. While the inflorescence is still closed, the lateral axes rest in these grooves, permitting the entire arrow to be squeezed into a very compact space.

The main axis carries lateral axes of the first order, and these in turn lateral axes of the second and third orders. The degree of branching decreases toward the top, and the upper part has only whorls of single axes. However, if these axes are branched, the secondary axes are inserted in such a manner that the joints of primary and secondary axes are grown together. (Fig. 2, A.) The lateral axes of the first order are longest and most profusely branched. The lower ones are far apart and arranged in semiwhorls, and the upper ones are close together and form complete whorls. The lateral axes of the second order and all primary axes with the exception of the lower ones begin with a pair of spikelets of which one is sessile and the other stalked. (Fig. 1, B.)

The developing inflorescence remains for a long time inclosed in a tube formed by the sheaths of the upper vegetative leaves. When the floral leaves become externally visible, the cane is spoken of as "boenting," a convenient term adopted from the Malayan language, for which there is no adequate English equivalent. Several weeks after the first indication of boenting, the flag (fig. 1, C) appears, and the inflorescence, which has a silvery hue on account of the silky hairs of the spikelet, is pushed out and expands. At the base of each lateral axis is present a cushionlike swelling, which enables the lateral axes to unfold and the inflorescence to open completely.

ANATOMY OF THE FLORAL AXIS

As a continuation of the apical growing point of the stem, the inflorescence axis shows a similar anatomical structure. A cross section shows numerous vascular bundles embedded in parenchymatous tissue. The central part is devoid of bundles and is often hollow. At the periphery the bundles are so small and so close together that they form a practically solid ring. The vascular tissue is separated from the epidermis by a narrow cortex, the cells of which are thick walled and heavily lignified. The epidermis itself consists of long

⁵ Credit is due Mrs. E. Artschwager for preparation of the colored plate and all other drawings in this publication.

narrow cells with sinuous walls and alternating short cells. At the insertion points of the lateral axes there are cushionlike swellings

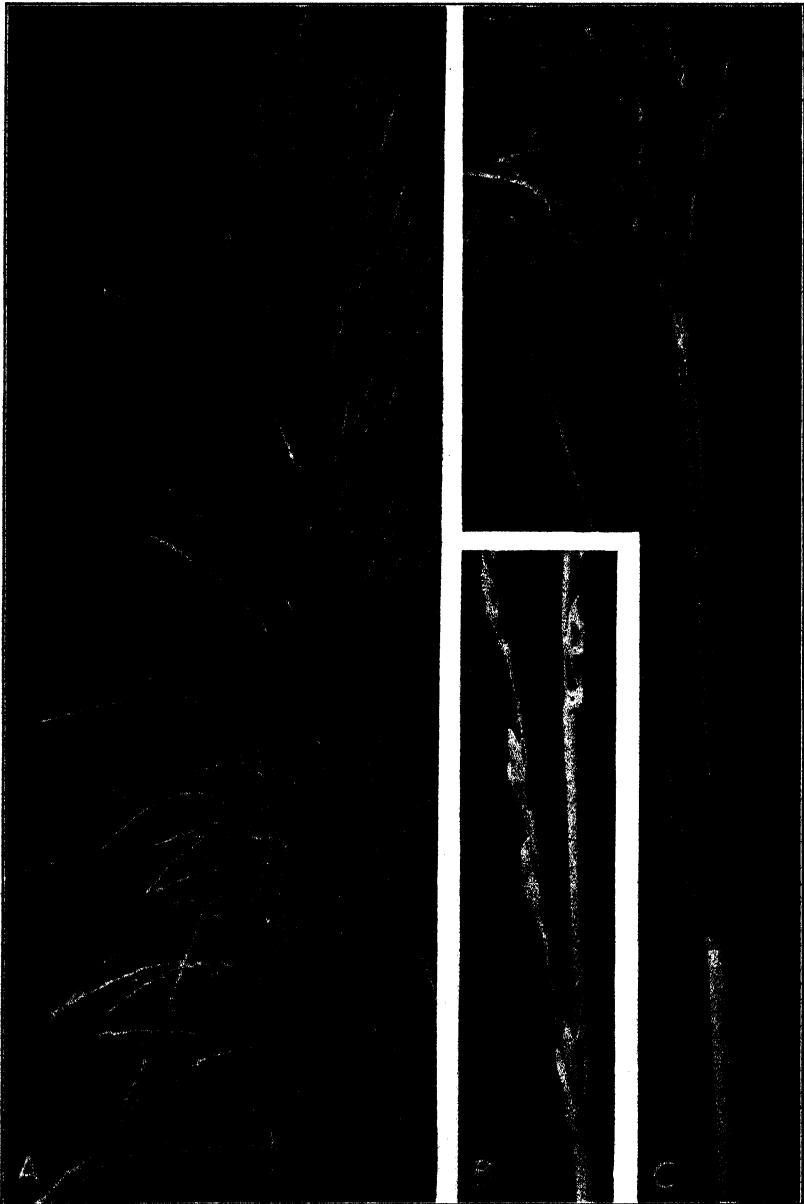


FIGURE 1.—A, Inflorescence of sugar cane fully expanded; B, lateral axis of inflorescence bearing pairs of spikelets; C, inflorescence in the "flagging" stage

made up mostly of parenchymatous tissue with the vascular elements limited to the abaxial side of the organ. This special modification

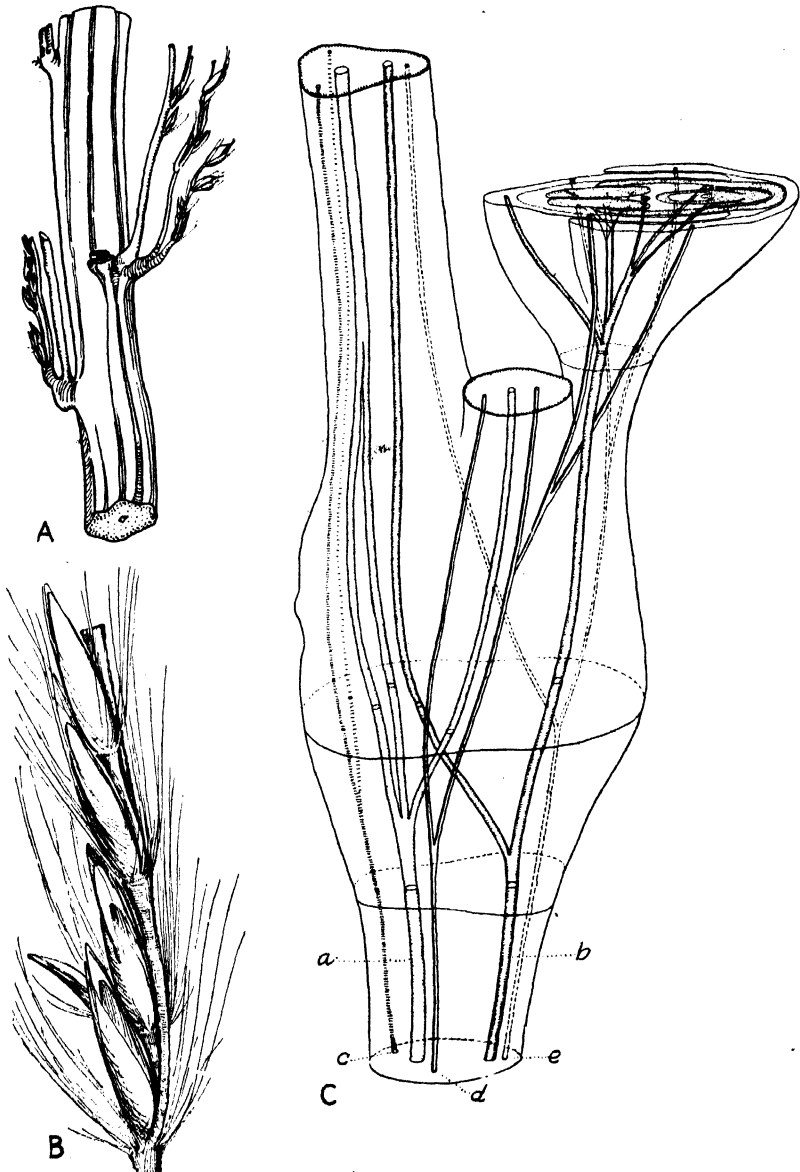


FIGURE 2.—A, Lateral axes arising from the same cushion joint of upper part of main axis. B, Part of rachis of inflorescence. Each rachis articulation bears a pair of spikelets of which one is sessile and the other stalked. C, Diagram illustrating the course of the vascular system of the rachis, the pedicel, and the sessile flower; *a* and *b* represent the two large bundles of the rachis and *c*, *d*, and *e*, the three small peripheral bundles

enables the lateral axes to expand, and at the same time it gives a greater flexibility to the joints and prevents injury during abnormal weather conditions.

The lateral axes of the first order are very much thinner than the main axis. The basal part is flat triangular, becoming more or less terete higher up. Like the main axis, it is traversed by a number of vascular bundles of which the inner ones are large and the peripheral ones small and surrounded by thick-walled lignified parenchyma. The lateral axes of the higher order are more or less flat triangular and possess a varying number of vascular bundles. The rachis, which bears the spikelets and which may be an axis of the first, second, or higher orders, possesses from four to six bundles, of which three are small and the remaining one to three much larger. The stalk of the pedicellate spikelet differs from the rachis in being thinner and possessing only three bundles, of which two are small and the third one much larger.

A transverse section of the rachis below the cushion joint shows a stele of five bundles and from these bundles there pass off a number of traces to supply the floral parts of the sessile flower and of the pedicel. (Fig. 3, A-H.) Above the point of departure of these traces the rachis continues, possessing the number of bundles it contained below.

The rachis joint, where the branching of the bundles occurs, differs from the rachis in being round and possessing a much greater diameter. (Fig. 3, A.) The cells of the parenchyma tissue are small and very short. They have greatly increased, forming a broad band between the more or less centrally located vascular strands and the epidermis. Many cells of the latter have enlarged and grown out into lignified hairs. Above this zone are several tiers of epidermal cells conspicuous on account of their very regular radially elongated form and the dense protoplasmic content. Just beneath the epidermis is found a sclerenchymatous hypodermis one cell wide.

A little distance below the cushion joint the two larger bundles (fig. 2, C, *a* and *b*) break up into three and two strands, respectively (fig. 3, A, B, C, D). Two strands of *a* and one of *b* continue into the next rachis segment, and the third strand of *a* passes out into the pedicel, while the second strand of *b* constitutes the larger part of the vascular system of the sessile flower. Simultaneously with the splitting of the two larger bundles the three small bundles of the rachis also divide. The two halves of each separate more or less and then extend into the various organs, as shown in the accompanying figures.

Frequently the rachis possesses three larger bundles instead of two and occasionally only one. When there are three large bundles, one extends into the next rachis segment without dividing, but if there is only one, two divisions occur, and the resulting three strands extend into the various organs, where they form the larger bundles. The behavior of the small bundles is in all cases more or less identical.

The outer glume of the flower is traversed by four and sometimes five or more bundles, and the inner glume is keeled and contains commonly three vascular strands. The sterile lemma has frequently a midrib, but the fertile palea and the fertile lemma, if present, have no vascular elements. The thick hyaline lodicules each contain a number of procambial strands. Each stamen has a single bundle. The remaining vascular tissue splits into three strands, which traverse the tissue of the ovary wall and converge in the style.

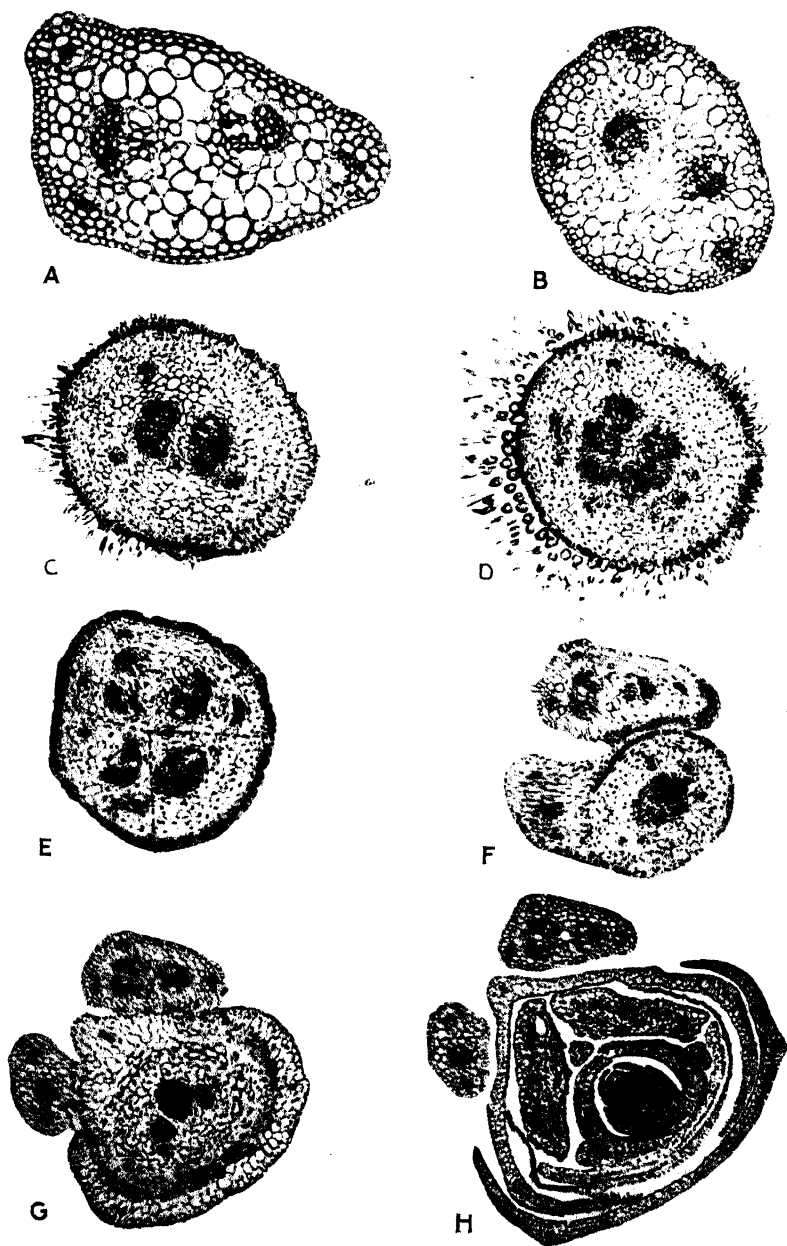


FIGURE 3.—A-H, Series of cross sections through a rachis joint (cf. fig. 2, C), illustrating the course of the vascular system of the rachis, the pedicel, and the sessile flower in the U. S. 875 variety. A, $\times 165$; B-H, $\times 67$

THE SPIKELET

The rachis (fig. 2, B) to which the flowers are attached is articulate and very brittle. Each rachis segment is narrow at the base and broader at the apex, one side of it being more or less convex and the other flattened and slightly concave.

At the nodes of the rachis are alternately placed two spikelets, of which one is sessile and the other stalked. Both spikelets are oblong lanceolate and uniflorous and have tufts of long silky hairs at their bases.

Each flower (pl. 1, A, and fig 4, A) is subtended by two bracts which form the outer and inner glumes. Inside the outer glume is found a

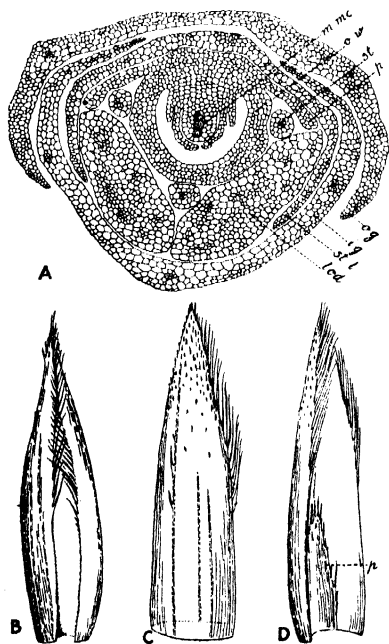


FIGURE 4.—A, Cross section of a young flower of U. S. 1694: *m mc*, Megaspore mother cell; *o w*, ovary wall; *st*, stamen; *p*, palea; *o g*, outer glume; *i g*, inner glume; *st l*, sterile lemma; *lod*, lodicule. $\times 80$. B, Outer glume. C, Inner glume. D, Sterile lemma inclosing the palea (*p*)

sterile lemma (third glume), inclosing in its turn the fertile palea. The fertile lemma (fourth glume) was wanting in the varieties of *Saccharum officinarum* examined, but was present in the hybrids of *S. spontaneum*. The sterile palea is always wanting. At the base of the flower, just inside the inner glume, are found two thick hyaline lodicules. Lastly, the axis bears a whorl of three stamens and the ovary.

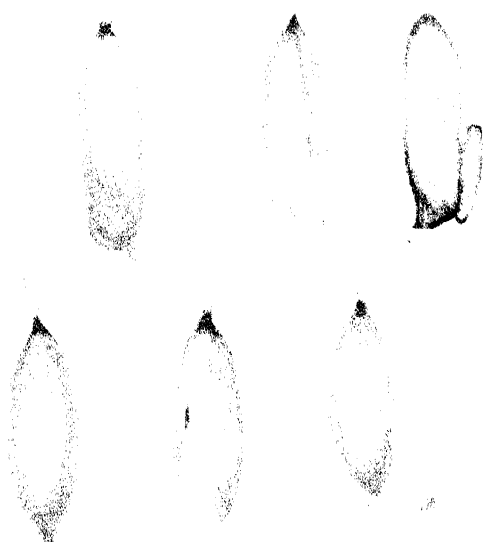
The outer glume of the spikelet (fig. 4, B) is bicarinate with slightly arched back and embraces with its edges the inner glume. The two keels and the edges are traversed by vascular bundles, which may extend to the tip of the glume. Occasionally a weakly developed midrib is present. The upper two-thirds of the glume is covered with short pointed hairs. The keels possess stronger hairs than the lamina between, and the edges are covered with lashes composed of long unicellular hairs. From the foot of the glume, the callus, arises a whorl of long silky hairs, several times the length of the spikelet.

The inner glume (fig. 4, C) is, like the outer, lanceolate pointed, but it possesses a well-developed midrib. On either side of the midrib is found a small vascular bundle. The back of the glume is also covered with short, closely adhering hairs, but the lashes along the edges are slightly longer.

The sterile lemma (fig. 4, D) is much more delicate than the two glumes, and it is commonly devoid of vascular bundles. It is a little shorter than the inner glume. The tip is slightly blunt in the varieties examined, but according to Jeswiet (13) it can be either blunt or pointed. The edges of the sterile lemma are covered with long lashes, which are especially prominent near the middle. The fertile lemma, which was found only in the *Saccharum spontaneum* hybrids, is a



A



B

U. S. 730, 1930, 1931

A.—Open sugarcane flower of variety U. S. 730; *i. gl.*, inner glume; *a. gl.*, outer glume; *s.*, stigma; *st.*, stamen; *fl.*, filament of anther; *o.*, ovary; *a.*, anther; *l.*, lodicules; *p.*, pubes; *st.*, sterile lemma; *fl.*, fertile lemma; *o.*, ovary; *s.*, stigma; *a.*, anther; *fl.*, filament of anther. $\times 15$.

B.—Stages in the germination of sugar-cane seed. $\times 15$.

narrow scale covered at the tip with hair or lashes and is devoid of vascular bundles.

The fertile palea is also small (fig. 4, D, *p*) and very irregular. Its edges are clothed with long hairs, and a few short hairs are found on the upper surface of the lamina. It is, according to Wilbrink (18) and Jeswiet (13), very changeable regarding shape and pubescence, but since these characters are constant in different forms, it serves in the study of taxonomic relationships.

The lodicules (fig. 5, E) are very short, more or less wedge shaped, with saucer-shaped hollow tops. They possess short projections on the upper edges, which, in the case of the *Saccharum spontaneum* hybrids, may elongate into longer or shorter lashes. During the time of flowering the lodicules swell greatly and push the glumes wide apart (fig. 5, E) so that the anthers and the pistil can protrude.

THE FLOWER

The sugar-cane flower is hypogynous and simple in structure, consisting of a whorl of three stamens and a single carpel. Often the flowers are imperfect, the ovary being rudimentary and, what is more often the case, the anthers without pollen grains.

The number of stamens is three; one filament is inserted between the lodicules and the other two on the opposite side of the flower, resting against the fertile palea. (Fig. 4, A.) The filaments are white and the anthers yellow and later purple. If they remain yellow or become papery they are invariably sterile. The anthers are bilobed and versatile, the filaments being attached to the connective near the base of the anther lobes. Each lobe contains two loculi (fig. 6, D), and the tissue that is between breaks down at the time of dehiscence. The filament before the opening of the flower is terete. It consists of elongated parenchymatous cells surrounding a small centrally located vascular bundle. When flowering occurs the filament grows very rapidly, thereby becoming irregular in thickness. The tissue of the connective is parenchymatous with a small bundle similar to that in the filament. The epidermis of the lobes is composed of narrow cells covered by a thin cuticle. Next to the epidermis is the endothecium composed of narrow cells with their long axes arranged at right angles to those of the epidermis. Shortly before dehiscence they develop thickening bands.

In the literature on sugar cane, references to the structure of the flower are mostly fragmentary. Of more than passing interest is the contribution of Cobb (7).

The gynecium (fig. 5, A) consists of a single carpel with an obovate or obconical ovary round in transverse section and flattened on the ventral side. The walls of the ovary consist of delicate parenchyma cells. Running longitudinally through it are two very fine lateral vascular strands. From the tip of the ovary arise two terminal styles which curve outward when flowering occurs. (Fig. 5, B.) Within the ovary is a single ascending anatropous ovule attached by a broad placenta on the inner ventral surface of the ovary. It possesses two integuments. The outer one, which disintegrates when the seed is ripe, is much shorter than the inner one and never reaches the micropyle. Each integument has a double layer of cells, but in the region above the placenta and at the micropyle it consists, as already shown by Guérin (10), of three or four layers.



FIGURE 5.—Sugar-cane variety U. S. 1694: A, Gynecium, $\times 17$; B, flower about to open, $\times 9$, showing protruding tips of the style; C, longitudinal section of a pair of young spikelets, $\times 110$, the stalked spikelets being advanced in development over the sessile one; D, longitudinal section of an older spikelet, $\times 110$; E, longitudinal section of an expanded flower of U. S. 1639, $\times 50$, the lodicules having forced the flower open through their ability to swell

DEVELOPMENT OF SPIKELET AND FLOWER

The stalked spikelet starts development before the sessile one. It shows already the developing glumes and hairs, while the sessile spikelet is just beginning to differentiate from the primordium. (Fig. 5, C.) The sessile spikelet develops at the inner side of the

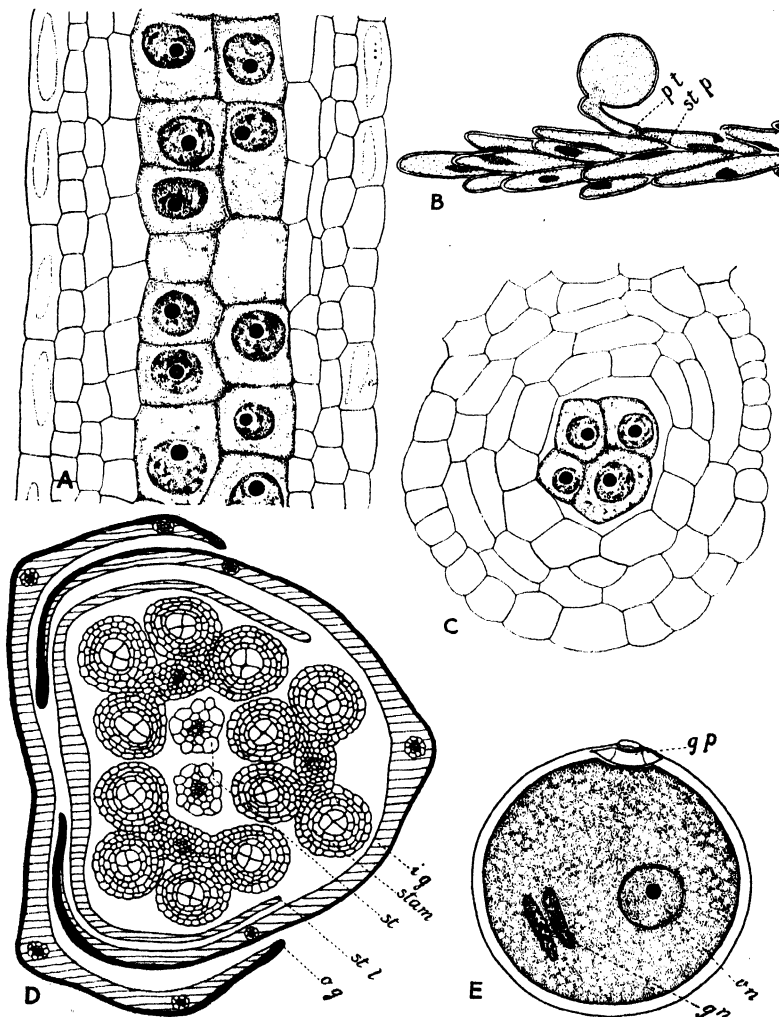


FIGURE 6.—Sugar-cane variety U. S. 1694: A, Longitudinal section of an anther with pollen in mother-cell stage, $\times 680$; B, lateral branch of feathery stigma with germinating pollen grain, $\times 272$; pt, pollen tube; st p, stigmatic papilla; C, cross section of an anther with pollen in mother-cell stage, $\times 680$; D, cross section of a young flower: i g, inner glume; stam, stamen; st, style; st l, sterile lemma; o g, outer glume; $\times 110$. E, mature pollen grain; v n, vegetative nucleus; g n, generative nuclei; g p, germ pore; $\times 1224$

base of the pedicel, and for this reason there are four rows visible at the convex side. (Fig. 2, B.) Only a little of the sessile spikelet is seen at the back side, so that there is a conspicuous dorsiventrality.

A detailed description of the development of the grass flower, especially of the androecium and gynecium, is given by Golinski (9)

and Koernicke (14), and with it the description of the sugar-cane flower, as given below, tallies. The floral organs and the bracts by which they are inclosed arise in succession as bulging masses of meristem upon the periphery of the primordium, beginning with the lower glume and ending with the carpel. (Fig. 5, D.) Each begins as a ridge of tissue that extends part way around the primordium of the flower. The small fertile palea and the lodicules arise at the same time and from the same rudiment. They are at first triangulate but gradually acquire the oblong shape characteristic of the mature organ. The stamens arise as conical outgrowths around the periphery of the apex of the flower. They develop early, the filament appearing before the lamina of the lower glume is differentiated and while the carpel is still small. A constriction which indicates the beginning of the filament occurs very early at the base of the stamen. The subsequent growth of the stamens until the pollen is ripe is due almost entirely to the elongation of the anthers, the filament growing only immediately before the pollen is shed. The carpel is the last of the floral organs to arise. It appears as a single ridge of tissue on the interior side of the floral axis and gradually encircles the apex of the young flower. Soon after it has completely surrounded the tip of the flower axis, two protuberances appear on the sides and form the beginnings of the stigmas.

MICROSPOROGENESIS

It is not within the compass of this investigation to treat in detail the early stages of karyokinesis, for it would lead to a consideration of the reduction problem in general. The sugar-cane flowers, moreover, are not suited for such a study, since the nuclei are comparatively small and the division figures, especially the early prophase stages, are complex and minute in structure.

Dividing pollen mother cells are found in inflorescences that have just begun to flag. They appear naturally first at the top of the inflorescence and tips of the lateral axes and progress centripetally and downward as the panicle expands. According to Bremier (4), this difference in the appearing of the pollen mother cells at the top and at the base amounts to six days in *Saccharum officinarum*, but to only four days in *S. spontaneum*. The young pollen mother cells are seen in longitudinal sections of the anther (fig. 6, A) in two rows fitting closely into one another and bordered by tapetum cells. In cross sections of the anther one observes usually four pollen mother cells arranged in such a manner that they all touch the tapetum with one of their sides. (Fig. 6, C and D.)

The young pollen mother cells contain only a little chromatin, which is present in the form of small grains. As the cells increase in size the chromatin grains enlarge, become more numerous, and appear connected with one another by fine threads. Gradually the nuclear mass assumes a unilateral position and the chromatin threads exhibit the characteristic, but in this case indistinct, prosynaptic pairing. Contraction increases until the spireme shows the typical klumpen. Chromatin threads are then thrown into the nuclear cavity until the whole space becomes filled with the chromatic filament. (Figs. 7, B; 8, A; and 9, A.) The threads of the spireme, being double, appear to be thicker than the prosynaptic ones. The spireme is soon after segmented; the individual parts continue to

shorten and thicken until finally in the diakinetik stage (figs. 8, B, and 9, B) the bivalent chromosomes are formed. The number of paired chromosomes or gemini is very large compared to the size of the nuclear cavity, and it is impossible to count them accurately. Shortly after the formation of the bivalents the spindle fibers begin



FIGURE 7.—Sugar-cane variety U. S. 1694: A, Longitudinal section of anther with pollen in metaphase of the heterotypic mitosis, $\times 520$; B, prophase stage of the heterotypic mitosis, $\times 840$; C, pollen tetrads, $\times 750$

to differentiate, while the nuclear wall disappears. The shortening and thickening of the gemini continues until the metaphase when the chromosomes become almost isodiametric and deep staining. The chromosomes are now seen on the equatorial plate of the bipolar spindle. (Figs. 7, A and 8, D.) The polar view of the metaphase (figs. 8, C, and 9, C) is especially well suited for the counting of the

chromosomes. It was found that in the U. S. 875 variety the number of chromosomes was 58. Since this variety is a Kassoer seedling, it should, according to Bremer (4), possess 68 chromosomes. The smaller number might be accounted for by assuming that the material that was used in this investigation came from a cross between U. S. 875 and another variety, or that the hybrid, by subsequent selfing, suffered a reduction in the number of its chromosomes. In the other

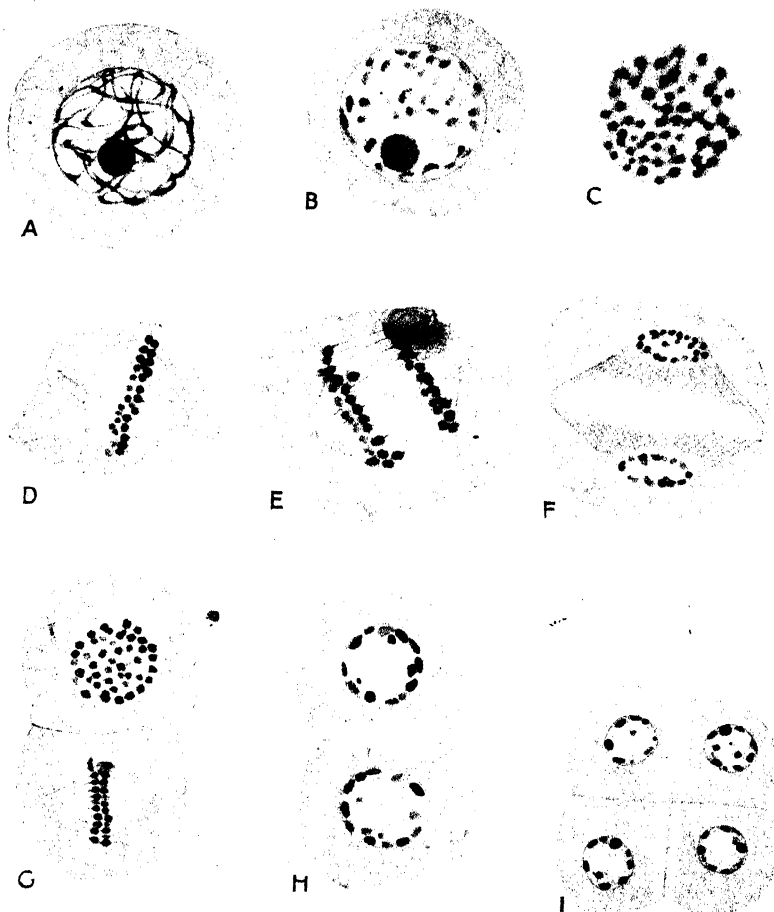


FIGURE 8.—Sugar-cane variety U. S. 785 at various stages in microsporogenesis, $\times 1140$: A, Hollow spireme; B, diakinesis; C, polar view of metaphase; D, metaphase; E, anaphasic separation; F, telophase; G, homotypic metaphase; H, diads; I, tetrads

variety, U. S. 1694, the number of chromosomes counted was 40, and since it is a P. O. J. 213 seedling this number is theoretically correct. The chromosome number of 14 which Franck (8) found for *Saccharum officinarum* does not agree with the haploid number of 40 found by Bremer (4) which was verified in this investigation.

While metaphase stages persist for a comparatively long time, the anaphasic separation (fig. 8, E) is quickly accomplished and appears to be quite normal, since there was practically no lagging of chromo-

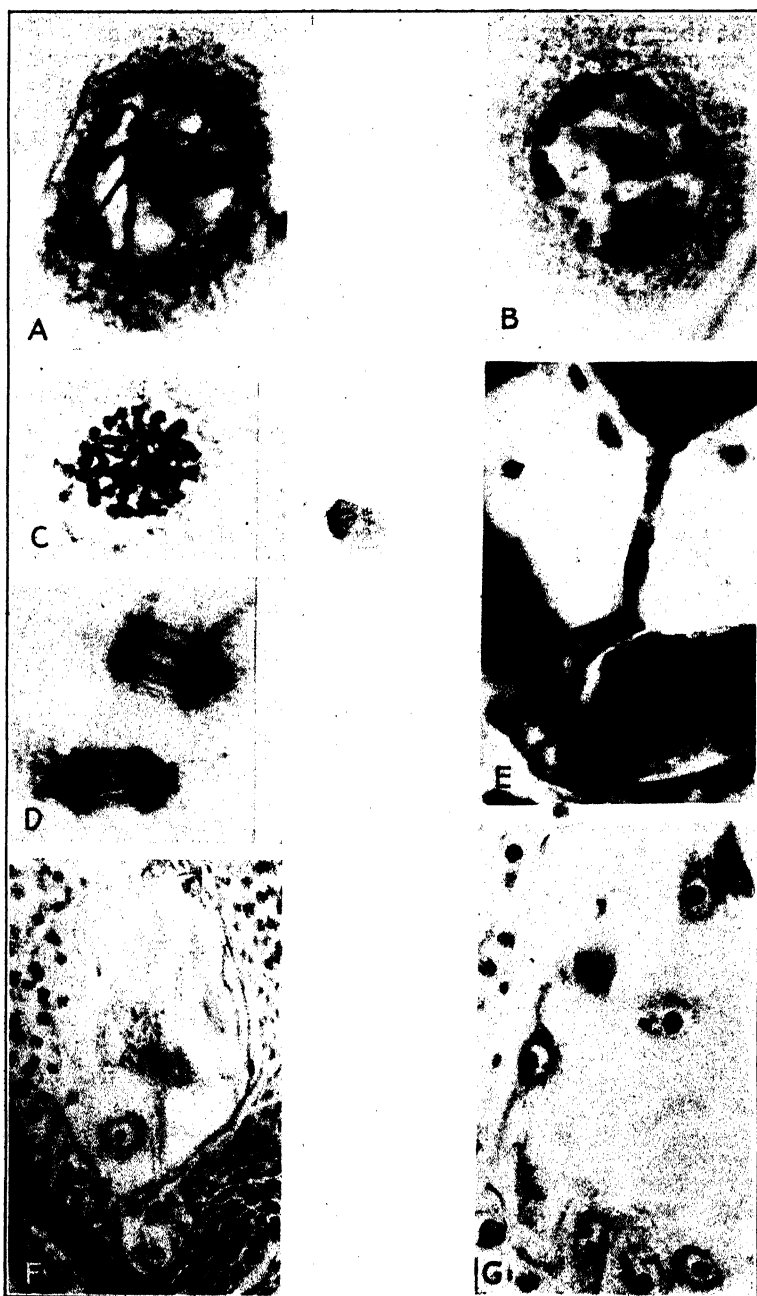


FIGURE 9.—A, Prophase stage (hollow spireme) of the heterotypic mitosis in pollen formation, $\times 1330$; B, diakinesis, $\times 1330$; C, polar view of metaphase of U. S. 875, $\times 1330$; D, anaphase of endosperm nuclei of U. S. 1694, $\times 740$; E, pollen tube penetrating micropyle of U. S. 1694, $\times 740$; F, embryo sac with fertilized-egg and endosperm nucleus in anaphase, U. S. 1694, $\times 215$; G, endosperm nuclei lying free in the embryo sac, U. S. 1694, $\times 230$

somes. At telophase (fig. 8, F) the chromosomes form a dense mass so that their number at this stage can not be ascertained. Just as in other monocotyledons, the two daughter nuclei are separated by a wall. (Fig. 8, H.) There is a short interkinesis during which the chromosomes retain their distinctness. In the homotypic division the nuclear spindles are usually arranged with their longitudinal axes parallel to one another, though occasionally they cross at right angles. (Fig. 8, G.) The chromosomes have the same isodiametric appearance, but they are smaller than those met with in the heterotypic division figures. The tetrad soon breaks up (figs. 8, I, and 7, C) into four microspores, which become invested by a wall that at first is thin and delicate. The extine of the mature pollen grain is thick (fig. 6, E), smooth, and possesses a single pore. It agrees essentially with the figures published by Schacht (17). In the protoplasm of the pollen grain are embedded three nuclei. The two generative nuclei are elongated and possess numerous dark-staining chromatin granules. The vegetative nucleus is spherical and has a large spherical nucleolus, but it stains only weakly compared to the generative nuclei.

While the pollen mother cells undergo the two divisions and the pollen is developing, changes occur in the various layers of the anther wall. The cells of the middle layer that have remained small become stretched tangentially and are soon obliterated. The cells of the tapetum are densely filled with protoplasm and contain at first a single nucleus. At about the time the sporogenous cells enter synapsis the nuclei of the tapetum divide and the cells become typically binucleated. At the time of the formation of the tetrads the tapetal cells show signs of disorganization; they separate and the content breaks down and is absorbed by the developing pollen. The cells of the epidermis and of the endothecium enlarge with increase in the size of the anther, and shortly before the dehiscence the cells of the endothecium develop thickening bands.

MACROSPOROGENESIS

The young ovule is atropous and consists first of a hemispherical mass of tissue derived from the morphological apex of the floral axis. Soon, however, as a result of differential regional growth it curves and becomes anatropous. During this change in position of the ovule the ovary increases greatly in size. It is flattened at first, becomes spherical, and finally oblong.

The archesporium is of subepidermal origin. It is wedge shaped (fig. 10, A) and at the time when both integuments of the ovule are initiated it is easily made out by reason of its relatively large size. It occupies over one-third of the nucellus and is eight times the size of the surrounding cells. (Fig. 10, B.) It keeps on enlarging until the ovule has both integuments fully developed. Then it divides rapidly twice, forming a tetrad row of megasporocytes. The outer three cells are gradually disorganized and remain for a time as deeply staining masses which finally disappear altogether. (Fig. 11, A.) The surviving mother cell enlarges and, pushing apart the cells on the sides of the narrow channel left by the disintegrating megasporocytes, comes in contact again with the epidermis.

The development of the embryo sac to the 8-celled stage follows the usual course. (Fig. 12, A-E.) The functional megaspore nucleus

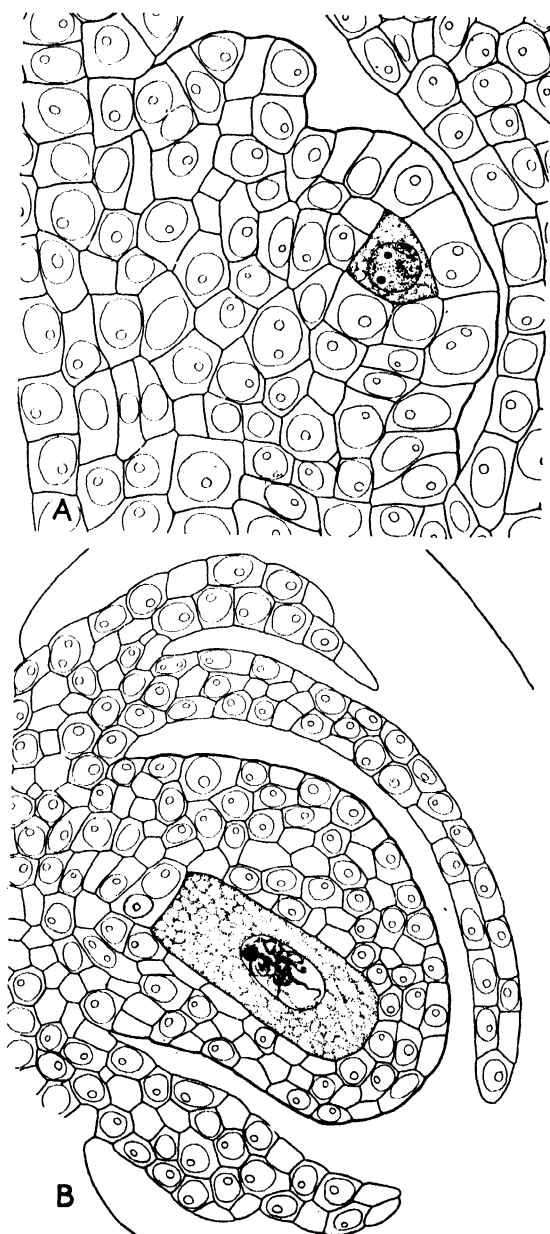


FIGURE 10.—A, Young ovule with megaspore mother cell differentiating, $\times 650$; B, ovule with megaspore mother cell greatly enlarged and integuments already developed, $\times 650$

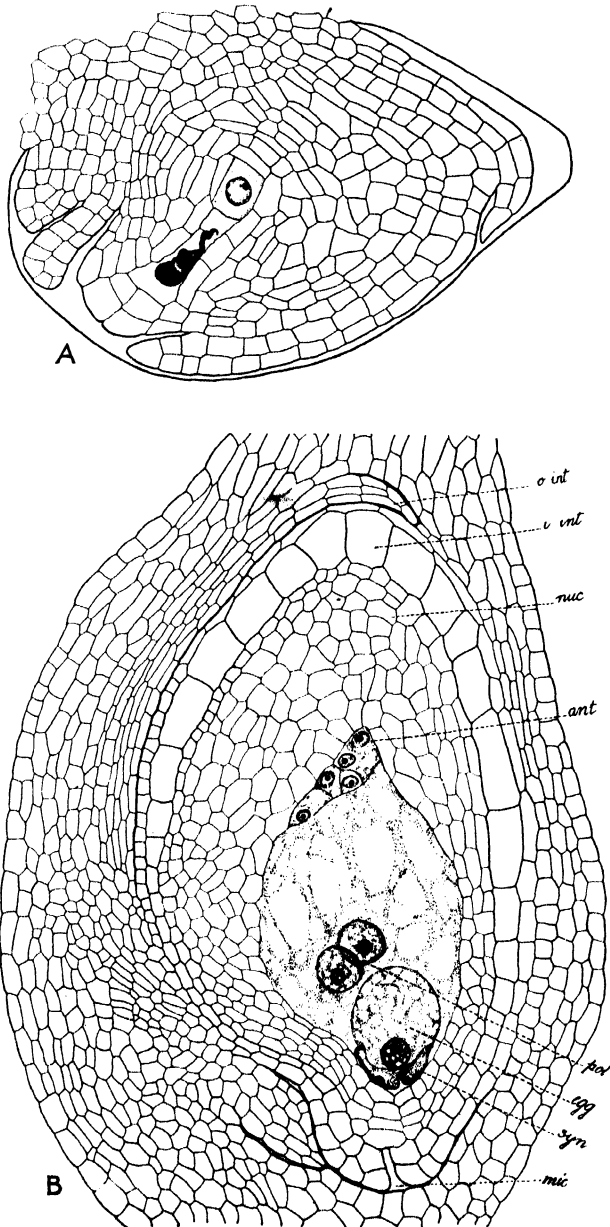


FIGURE 11.—A, Ovule with row of megaspore tetrads of which three are degenerated, $\times 250$; B, ovule with 8-celled embryo sac, $\times 250$: *o int*, Outer integument; *i int*, inner integument; *nuc*, nucellus; *ant*, antipodals; *pol*, polars; *egg*, egg; *syn*, synergids; *mic*, micropyle

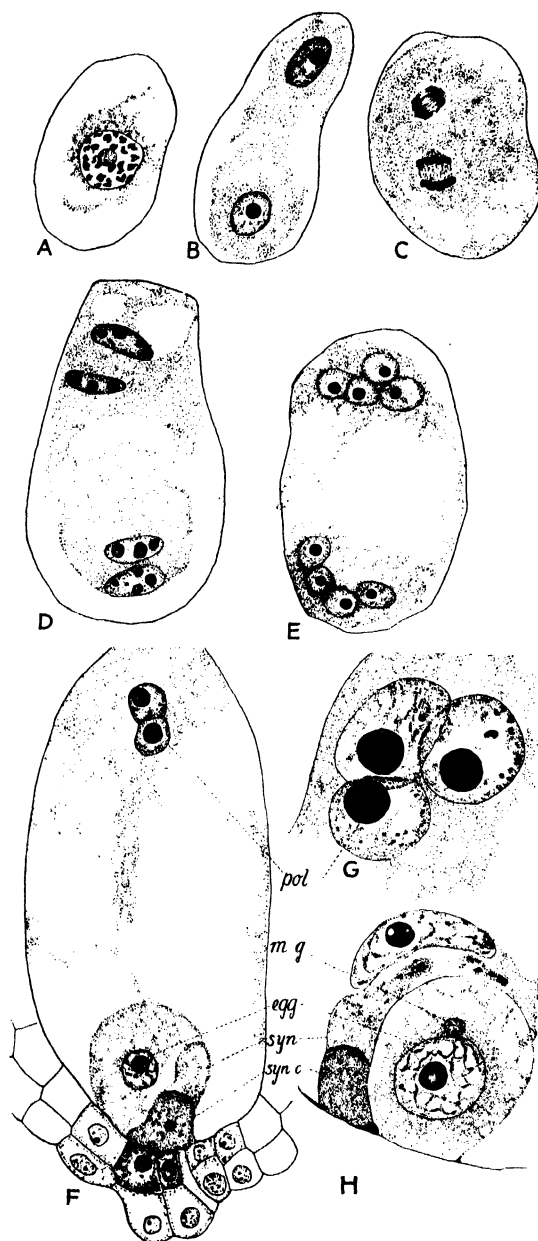


FIGURE 12.—Sugar-cane variety U. S. 1694: A-E, Different stages in the development of the 8-celled embryo sac, $\times 600$. F, An unusual type of embryo sac in which the polars are far removed from the egg, $\times 288$. For legend see Figure 11, B. G, A group of three polar nuclei in mature embryo sac, $\times 600$. H, Fertilization of the egg; the male gamete is seen inside the egg: *m g*, Male gamete; *syn c*, synergidal cap; *syn*, synergid, $\times 600$

divides into two nuclei, which lie one in the micropylar end and the other in the region of the chalaza. The chalazal nucleus divides at right angles, and the upper nucleus divides parallel to the long axis of the embryo sac. The nuclei of each group divide again. The sac now contains eight free nuclei. (Fig. 11, B.) At the micropylar end are two pear-shaped synergids with typical synergidal caps and a broader egg nucleus. The synergids do not readily take the stain, and their nuclei are rarely visible. Near the egg are the two polar nuclei, which remain in close contact with the egg and fuse to form the primary endosperm nucleus just before fertilization. Abnormalities in the development of the polars were observed on two occasions. In one preparation (fig. 12, F) the polar nuclei of an otherwise normal embryo sac were far removed from the egg and practically abut on the antipodals; in another preparation (fig. 12, G) there were observed three well-developed polars instead of two. The three nuclei at the chalazal end of the sac give rise to the antipodal complex. (Fig. 13, A.) The antipodal nuclei divide several times and each nucleus becomes inclosed in a globular mass of protoplasm. The antipodal complex shows in section frequently a characteristic crescent form. The nuclei vary in size (fig. 13, A); they often stain deeply; at other times they appear very faint. The disorganization of the antipodal nuclei begins simultaneously with the formation of the endosperm.

The mature embryo sac is covered at the apex by about two layers of nucellar tissue, the central ones somewhat elongated so that the apex of the nucellus is slightly pointed. (Fig. 9, F.) Within the embryo sac the granular cytoplasm forms an intricate network and is especially dense around the two polar nuclei. A longitudinal sagittal section of the mature embryo sac is shown in Figure 14, A. The sac is narrow in the upper part, gradually broadening toward the region of the micropyle. In sections cut at right angles to the sagittal the sac appears uniformly narrow. (Fig. 14, B.)

While the embryo sac is developing the nucellar tissue becomes disorganized, its disappearance being most rapid in the micropylar region and the dorsal side of the ovule. The epidermis of the nucellus continues for a long time. Its position and the arrangement of the cells render it liable to be mistaken for the aleurone layer before the latter is distinctly separated from the endosperm. Later its cell content becomes disorganized and the layer disintegrates.

ANTHESIS OF FLOWERS AND POLLINATION

The anthesis of the flowers takes place in the early morning hours before daylight in the latitude of Canal Point, Fla., according to Brandes (3). Calvino (5) confirms this in work conducted at San Manuel, Cuba, and states that meteorological conditions greatly influence the opening time, the dehiscence of the anthers being especially delayed by the presence of dew. Flowers that are about to open are recognized by the protruding pistils from the still closed glumes. (Fig. 5, B.) The filaments of these flowers are meanwhile increasing in length rapidly while the lodicules begin to swell, gradually forcing the glumes apart. The flowers usually remain open for only a short time; the pollen is quickly shed and carried by the wind to the receptive stigmas of the flowers.

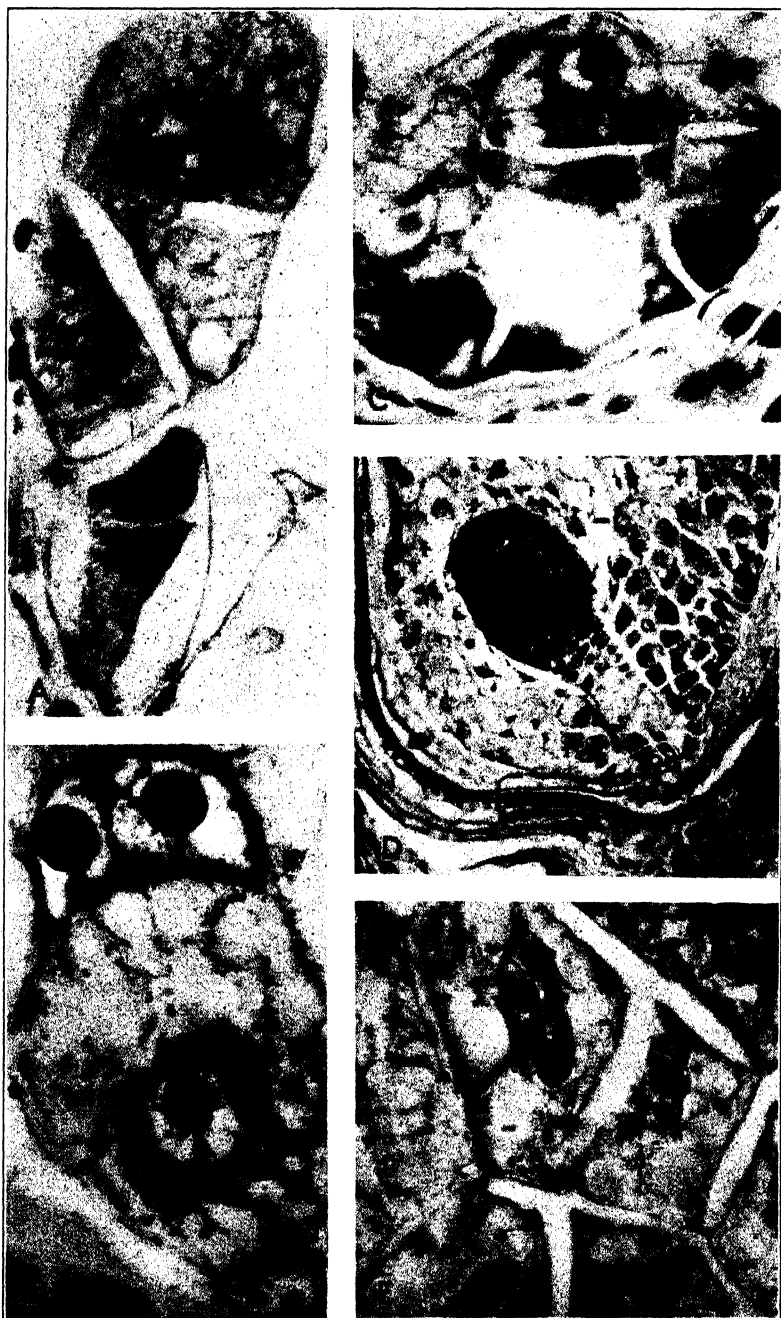


FIGURE 13.—A, Antipodal complex at time of fertilization of the egg, U. S. 1694 variety, $\times 850$; B, egg and polars at time of fertilization, U. S. 1694, $\times 850$; C, 3-celled embryo, U. S. 875, $\times 850$; D, older embryo, U. S. 875, $\times 220$; E, cellular endosperm, U. S. 875, $\times 850$

FERTILIZATION AND DEVELOPMENT OF SEED

The pollen, falling upon the receptive stigmatic papilla, germinates immediately. The pollen tube grows through the papilla (fig. 6, B) into a lateral branch of the stigma and thence downward until it reaches the conductive tissue of the style. Upon entering the ovary

cavity it grows between the outer integument and the ovary wall to the micropyle, pushing down between the cells of the nucellus (fig. 9, E), which appear to be injured by its passage. The further history of the pollen tube and the actual discharge of the male nuclei could not be followed in the material that was available for study, yet the presence of the male gametes in the egg cell (fig. 12, H) and the appearance of two nucleoli inside the egg nucleus (fig. 14, C) of another preparation indicate that fertilization has taken place and that the process may be taken as normal.

The egg enlarges somewhat after fertilization. (Fig. 14, B.) It is pear shaped, sometimes adhering with the narrow end to the micropylar end of the sac and sometimes possessing a broader base of attachment. In this condition it remains until the development of the endosperm is well advanced.

The polar nuclei at the time of fertilization are found in close contact with the egg.

(Fig. 13, C, and 14, A, B.) They fuse at the time the egg is fertilized or soon after and at once begin to divide rapidly. (Fig. 14, D.) The first nuclei are seen in the thin protoplasmic lining in different parts of the embryo sac. (Fig. 9, G, and fig. 15.) The nuclei differ

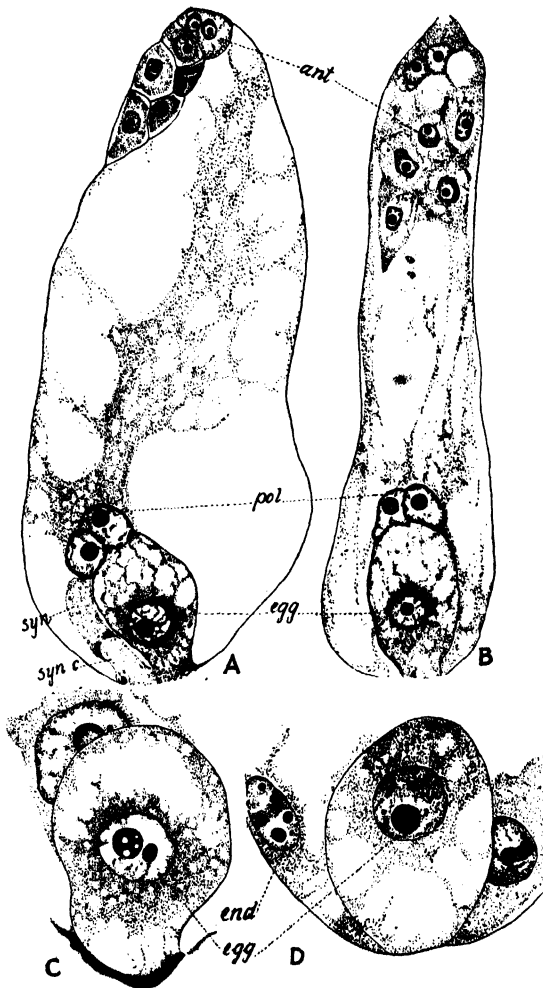


FIGURE 14.—A, Longitudinal sagittal section of embryo sac: *pol*, Polars; *syn*, synergids; *syn c*, synergidal cap, $\times 208$. B, Longitudinal radial section of embryo sac: *pol*, Polars, $\times 208$. C, Egg nucleus with two nucleoli, $\times 520$. D, Fertilized egg at rest surrounded by free endosperm (*end*) nuclei, $\times 520$. For legend see Figure 11, B

among themselves at all stages in the number of nucleoli which they possess. Division in the nuclei is at first simultaneous, since one observes the same division figures in all the nuclei of the sac. (Fig. 9, D.) With their increase in number they become larger in size, cease dividing, and become inclosed in rather dense masses of protoplasm that later becomes separated by cell walls. (Fig. 13, E.)

The first division of the fertilized egg is transverse and results in the formation of a smaller epibasal and a larger basal cell. (Fig. 15.) The second division occurs in the basal cell and is also transverse. (Fig. 13, B.) This sequence of divisions appears to be common in grasses, as shown by the studies of Nörner (16) and Cannon (6). The third division takes place in the epibasal cell, bisecting it parallel to the long axis of the embryo sac. The third division, however, may occur first in the cell below the epibasal, or it may occur almost simultaneously in both cells. Since most varieties of sugar cane produce even under favorable conditions only a small percentage of seed, it does not lend itself to embryonic studies. Although embryos in various stages of development were obtained (fig. 13, B, D), it was impossible with the material on hand to follow the sequence of division and to follow out the ontogeny of the different parts of the mature organ. Further work may disclose that failure of the fertilized egg to keep pace with the growth of the ovule and to form a viable seed is due to cessation of growth while yet in the proembryonic stage, or that it results from abnormalities in the reduction division, or unfavorable environmental conditions during the pollination period.

STRUCTURE OF THE SEED AND SEED COAT

The fruit of the sugar cane, like that of other grasses, is a caryopsis. It is narrow, ovate to oblong, and in the region of the embryo there is commonly visible a slight depression. At the proximal end is the scar of the seed, while the distal end often bears the remains of the style. (Pl. 1, B.) When immature the seed has a milky-white color, but when ripe it is a yellowish brown. The seeds vary considerably in size in spite of their minuteness, but it is of interest to note that the decrease in size is caused by a reduction in the amount of endosperm tissue and that the size of the embryo itself is more or less constant. Cobb (?) illustrates the structure of a mature seed with an excellent drawing of a cross section near the distal end of the embryo.

A median longitudinal section through the seed (fig. 16) shows, at the proximal end, the relatively large embryo, the position of which is marked externally by a slightly lighter color. The embryo is partly surrounded by endosperm tissue, and both embryo and endosperm are covered by the seed coat. The embryo possesses a short mesocotyl, at the apex of which is the growing point surrounded by rudimentary leaves and inclosed by the coleoptile; at the base is the radicle completely inclosed by the coleorhiza. Almost surrounding these organs is the scutellum. (Fig. 17, B, C.) It is expanded laterally into two wings and extends farther down than the root, from which it is separated by a distinct cleft. The epidermis of the back of the scutellum, which is in contact with the endosperm, consists of more or less columnar epithelial cells which secrete diastase for the conversion of the stored food of the endosperm. The plumule

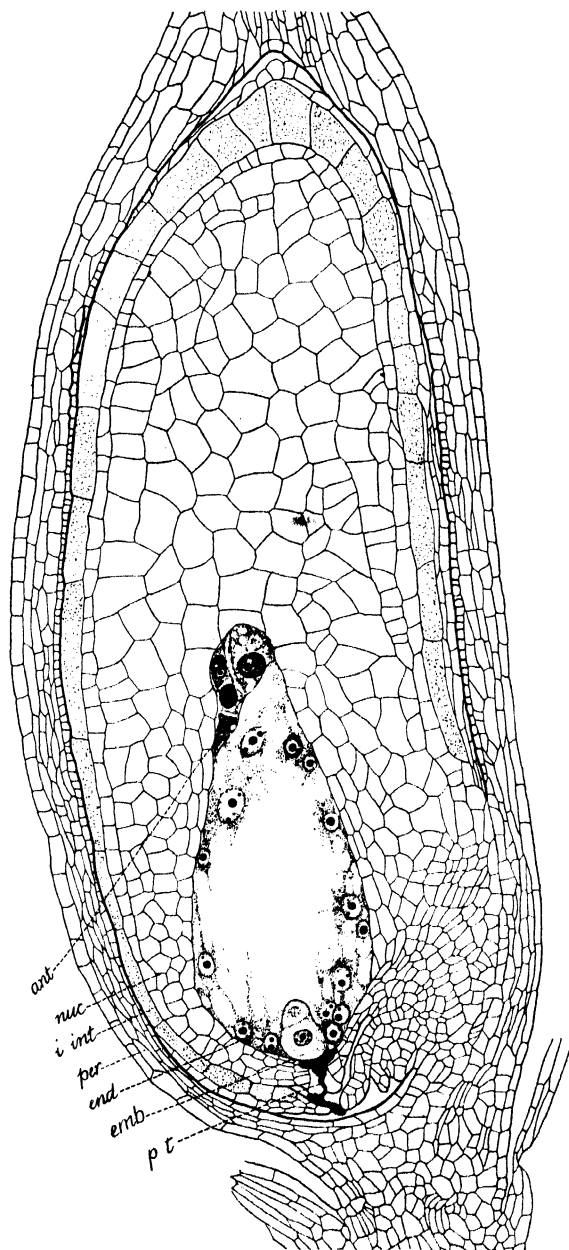


FIGURE 15.—Sugar-cane variety U. S. 1694. Longitudinal sagittal section of ovule with 2-celled embryo and numerous endosperm nuclei: *ant*, Antipodals; *nuc*, nucellus; *i int*, inner integument; *per*, pericarp; *end*, endosperm; *emb*, embryo; *p t*, pollen tube. $\times 160$

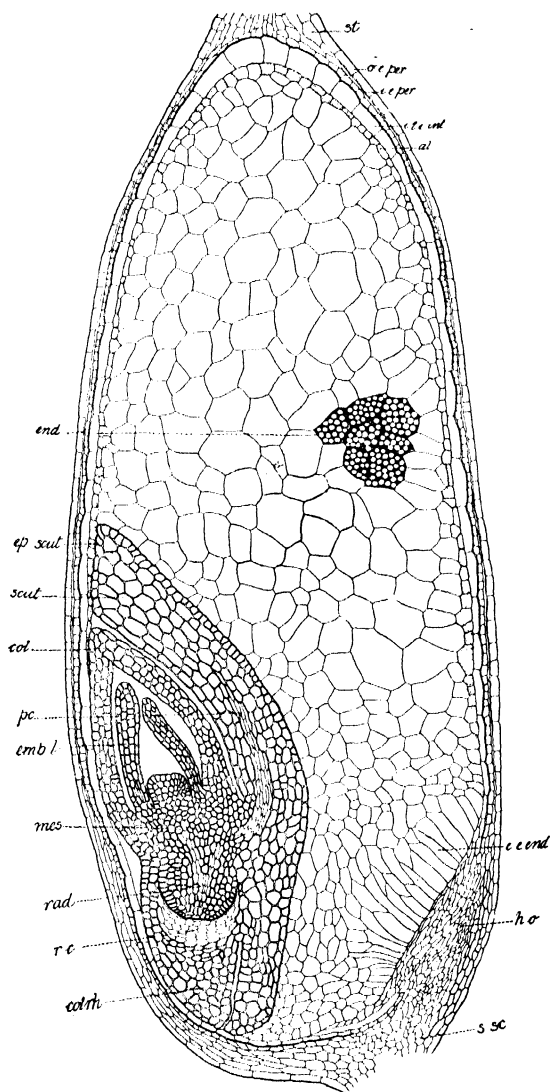


FIGURE 16.—Sugar-cane variety U. S. 1694. Sagittal section of a mature seed and embryo: *st*, Remains of style; *oe per*, outer epidermis of pericarp; *ie per*, inner epidermis of pericarp; *ili int*, inner layer of inner integument; *al*, aleurone layer; *end*, filled endosperm cells; *ee end*, empty elongated endosperm cells; *ho*, cells of hilar orifice; *s sc*, seed scar; *ep scut*, epithelium of scutellum; *scut*, scutellum; *col*, coleoptile; *pc*, procambium strand; *emb l*, embryonic leaves; *mes*, mesocotyl; *rad*, radicle; *rc*, root cap; *colrh*, coleorhiza. $\times 75$

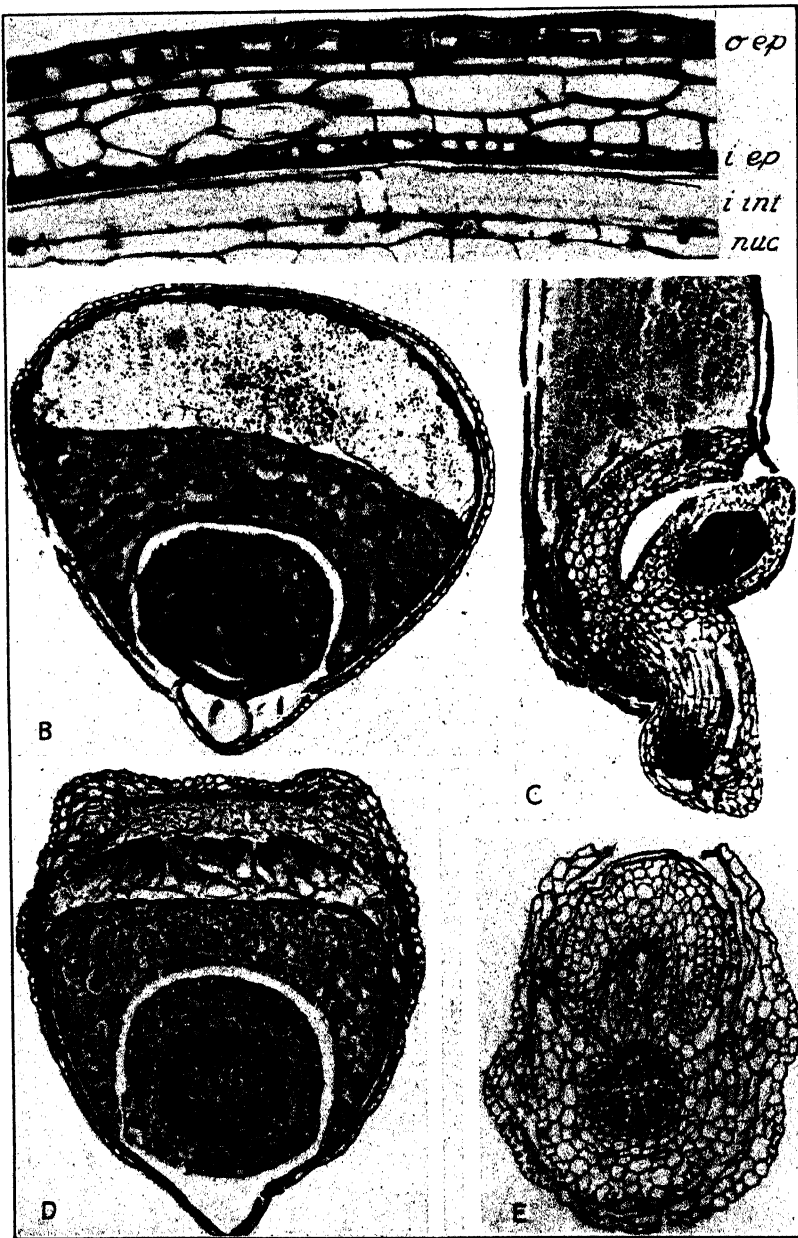


FIGURE 17.—Sugar-cane variety U. S. 1694. A, Ovary wall at time of fertilization: *o ep*, Outer epidermis; *i ep*, inner epidermis; *i int*, inner integument; *nuc*, nucellus, $\times 420$. B, Cross section of mature seed cutting through scutellum and tip of coleoptile, $\times 110$. C, Section through the same seed but at level of radicle, $\times 110$. D, Longitudinal section of germinating seed, $\times 46$. E, Cross section through root region of young seedling, secondary and primary roots are separating, $\times 70$.

consists of several rudimentary leaves surrounding the growing point. The radicle is covered by the root cap and is inclosed within the coleorhiza. The latter is a parenchymatous tissue connecting with the mesocotyl and the lower half of the scutellum.

The vascular system is present in the form of procambial strands consisting of elongated thin-walled cells. A broad procambial strand curves outward almost at right angles from the mesocotyl and extends upward in the scutellum. Upon the germination of the seed the procambial cells become differentiated into vascular elements. Simultaneously the epithelial cells elongate slightly and the endosperm cells next to the scutellum become devoid of starch.

Just inside of the seed coat and extending entirely around the embryo and the endosperm except in the region of the hilum and the micropyle is the aleurone layer. It is usually one cell thick, but occasionally several cells are superposed. The individual cells are rectangular in transverse and longitudinal sections and polygonal with rounded corners in surface view. In front of the scutellum the layer consists of smaller cells, while over the plumule and coleorhiza it becomes so thin and shriveled that it is difficult to recognize it as a separate layer. Each cell of the aleurone layer contains an oval nucleus with several nucleoli; the rest of the cell is filled with spherical aleurone grains embedded in an oily cytoplasm. In the region of the hilum and the proximal end of the scutellum lies a light-colored mass of elongated somewhat irregular empty endosperm cells. The endosperm tissue is made up of thin-walled polyhedral cells with those adjoining the aleurone layer smaller than the central ones.

The seed coat of the sugar-cane seed consists of the fused pericarp and the inner integument. The pericarp is made up of several layers of cells, of which only the outer and the inner epidermis are distinguishable. The outer epidermis is slightly undulated and consists of larger rectilinear cells with relatively thin walls. (Fig. 17, A.) The inner epidermal consists of thin-walled tubular cells, which lie parallel to the outer epidermal cells running from base to apex. (Fig. 17, A.) They are circular or broadly elliptical in cross section.

The inner integument forms a single layer of cells, continuous except at the hilum. (Fig. 16.) The cells are much larger than those of the pericarp. The walls are yellowish brown in color and the lumen becomes filled with a homogenous content. Toward the distal end these cells increase in size, and over the embryo they are thinner and lighter colored.

The micropyle is closed by the cells of the inner integument. The pericarp over the micropyle consists of a distinct inner and outer epidermis with three to four thin-walled cells between. The outer epidermis consists of thick-walled cells with large lumina and the inner epidermis of thick-walled pigmented tubular cells.

Since the seed never becomes detached from its pericarp, there is no true seed scar; however, there is a large opening in the inner integument in the position corresponding to the hilum. (Fig. 16.) Stretching over this region is a continuous stratum of several layers of pericarp cells with somewhat thickened walls. It is known as the closing tissue of the hilar orifice and has been described in detail by Harrington (11) for the seed of Johnson grass.

The germination of sugar-cane seed has been studied by various investigators, and Krüger (15) gives a series of drawings illustrating the stages in the development of the seedling. The material on which Plate 1, B, is based was furnished by B. A. Bourne, Office of Sugar Plants, who grew freshly harvested, hulled, and sterilized seed on agar cultures. He obtained the first indication of germination after 24 hours if he left the Petri dishes containing the seed exposed to the warm rays of the sun.

The primary root grows a few millimeters, develops a few stubby root hairs, and is then displaced by a secondary root. (Fig. 17, E.) This first lateral root as well as those following possess numerous root hairs, and the region of the root cap is usually colored red. After the emergence of the radicle the plumule elongates and the development of the seedling takes its normal course. A median sagittal section of a 36-hour-old seedling is shown in Figure 17, D. The radicle, although already broken through the pericarp, is still inclosed within the coleorhiza. The plumule became bent in the elongation process and was cut obliquely as seen in the picture. The cells of the epithelial layer of the scutellum have somewhat elongated, but there is no indication as yet of starch removal from the endosperm.

SUMMARY

Sugar-cane flowers are arranged in spikelets grouped in pairs, one sessile and the other stalked. Each flower is subtended by two bracts, which form the outer and inner glumes. Inside the outer glume is the sterile lemma inclosing in its turn the fertile palea. The fertile lemma is absent in most of the varieties of *Saccharum officinarum* but is present in the form of a thin, narrow scale in *S. spontaneum* and all its hybrids observed. At the base of the flower, just inside the inner glume, are two short hyaline lodicules. Lastly, the axis bears a whorl of three stamens and an ovary containing an ascending anatropous ovule.

The rachis possesses normally five bundles, two large and three small ones. These bundles split in the region of the rachis joint into a number of traces to supply the floral parts of the sessile flower and the pedicel of the stalked spikelet. One of the large bundles splits into three strands, of which two subsequently fuse again and continue into the next rachis segment, while the third strand extends into the pedicel. The second large bundle forks, one branch extending into the next rachis segment and the other forming the larger part of the vascular system of the sessile flower.

The stalked spikelet of the varieties studied starts development before the sessile one, but the difference gradually lessens so that at the time of fertilization both spikelets are equally far advanced.

Microsporogenesis in the varieties studied is normal. The chromosome number in U. S. 875, which is a Kassoer seedling, is $x=58$; the number in U. S. 1694, which is a P. O. J. 213 seedling, is $x=40$.

The megaspore mother cell is subepidermal in origin. It enlarges without previously cutting off a parietal cell and then divides twice to form the typical tetrad of megaspores, of which the innermost develops into the embryo sac. The latter is separated from the micropyle by several layers of nucellar tissue. Two cells of the outer layer of this tissue are especially large and serve as entrance for the pollen tube.

Both integuments of the ovule are in places more than two cells wide, especially in the region of the micropyle, but the outer integument surrounds the ovule only partly.

The antipodal cells increase in number and become in addition binucleated or multinucleated. Often their nuclei vary considerably in size. At the time of fertilization the antipodal complex degenerates.

The synergids are well developed but remain inconspicuous because of their poor staining reaction. The synergidal caps are well developed, but the nuclei of the synergids are seldom observed.

The polars which always closely adhere to the egg fuse at the time of fertilization, or soon after, and initiate free nuclear endosperm formation, which becomes cellular first in the region of the embryo.

The early development of the embryo does not differ from that in other grasses, the first two divisions of the zygote being transverse. The mature seed also possesses the typical structure of the grass Caryopsis.

The seed coat consists of the fused pericarp, of which, however, only the outer and the inner epidermis and the inner layer of the inner integument are recognizable.

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SULPHUR-SPRAY RESIDUES AND THE SWELLING OF TIN CANS PACKED WITH PEACHES¹

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INTRODUCTION

The varieties of peaches generally grown for supplying the fresh-fruit market are sometimes canned in considerable quantities, especially in years of overproduction. Owing to the nature of these varieties and to the warm summer temperature, the fruit ripens rapidly and becomes soft in a very short time after ripening begins. Notwithstanding prompt picking and handling, many peaches arrive at the canning plant too soft to be peeled with the usual lye-peeling machines. Such fruit is generally canned by being washed and packed in the usual way without peeling. Fruit packed in this way is sold as "pie peaches." Since the adoption of the sulphur sprays for the control of the brown-rot fungus, difficulty has been encountered with this material because of the swelling of the cans in a few months after canning. The extent of this loss varies greatly in different years.

At the request of some of the canners at Fort Valley, Ga., the writers undertook some work upon this problem in 1925 with the hope of gaining a better understanding of these difficulties.

LITERATURE

At the beginning of the work it was apparent that the swelling was due to spray residue, for no trouble had been experienced with lye-peeled material packed in the usual way.

Clough, Shostrom, and Clark (1)² had already shown that a lime-sulphur spray upon gooseberries caused gas formation when such fruit was packed in tin cans. The gas present was mainly hydrogen, but it was always accompanied by more or less hydrogen sulphide. They also found that the gas formation was considerably retarded when the fruit was canned in a heavy sirup.

Stevenson (9) has shown that lime-sulphur spray on cherries causes the swelling of the container when they are put up in plain tin cans. He has also shown that difficulty may be encountered in washing the spray residue from the fruit. He refers to one instance of swelling of cans of unpeeled pie peaches caused by lime-sulphur spray.

EXPERIMENTAL WORK

In 1925 and 1926 lots of fruit that showed the presence of adhering spray material were selected from the orchard. A quantity of fruit that had received no spray whatever was also obtained. The

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² Italic numbers in parentheses refer to "Literature cited," p. 40.

unsprayed material was canned without peeling, both with and without exhaust. One portion of the sprayed material was canned without being washed; another was canned after being washed in cold water; one was dipped in hot dilute lye (0.1 per cent was used, and at frequent intervals enough was added to keep the solution slightly alkaline) and then washed by being sprayed with jets of cold water; another portion was canned after being lye peeled in the usual way.

In the unsprayed unpeeled lots and in the lye-peeled lots that had been exhausted for four minutes no appreciable swelling occurred in two years. The unsprayed unpeeled lots and the lye-peeled lots that were not exhausted showed about 10 per cent of "flippers"³ at the end of two years. In the 1925 pack practically all the unpeeled sprayed material that was given an exhaust—that unwashed and that washed in cold water—swelled 8 to 14 months after canning. In the 1926 pack the lots thus treated required several months longer to develop swelling, and some had not swelled at the end of two years. The unexhausted cans of unpeeled sprayed fruit swelled very much sooner, some in a few weeks after canning, and nearly all swelled in two years. The lots washed in dilute lye and exhausted did not swell in two years. The unexhausted cans of fruit that had been washed in dilute lye contained about 10 per cent of "flippers" or "swells" at the end of two years; that is, they behaved about like the unsprayed unexhausted checks. It is clear that an efficient exhaust will greatly prolong the time required for swelling to occur.

It is very evident from these experiments that spray residue is rather hard to wash from the surface of peaches. This seems to be due to the insoluble nature of the spray residue and to the velvet-like covering of plant hairs upon the fruit. Washing with cold water and even with hot water did not seem to be entirely effective. Immersing the fruit in hot dilute lye for a few seconds and then washing with cold water has proved effective during the two years that this treatment has been used. It has been found possible to dip quite soft fruit into hot dilute lye for 15 to 30 seconds and then to remove the lye by spraying with jets of cold water without causing disintegration.

EFFECT OF VARIOUS FORMS OF SULPHUR

Since it is possible that the sulphur may exist upon the fruit in several forms, it seemed advisable to determine which form is responsible for the swelling of the cans. Accordingly, in 1925 five lots of unsprayed peaches were put up in No. 2 plain tin cans to which were added lime-sulphur spray, free sulphur as flowers of sulphur, hydrogen sulphide, sodium thiosulphate, and sodium sulphite, respectively. The quantity of fruit filled into the cans varied from 325 to 350 gm., and the quantity of water added varied accordingly, the total weight of the contents being approximately 540 gm. The lots of cans were divided into two series; one series received the equivalent of 100 mg. of sulphur per can, and the other received the equivalent of 10 mg. The sulphur was added as the fruit was packed in the cans. After the proper quantity of water had been added, the cans were exhausted and sealed. In the case of the hydrogen sulphide the addition was made subsequent to exhausting and just before sealing.

³ A "flipper" is a can slightly distended with gas; a "swell" is one distended more firmly or to a greater extent.

The cans containing lime-sulphur spray or hydrogen sulphide showed swelling first, whereas those containing sodium thiosulphate were the last to show swelling. The lots canned as checks contained less than 1 per cent of swells at the end of two years. The lots receiving 100 mg. of sulphur in all the above-mentioned forms swelled within two years; those receiving the equivalent of 10 mg. of sulphur—as hydrogen sulphide, sodium sulphite, or in the form of lime-sulphur spray—showed a few swells at the end of the first year of storage, and at the end of two years 10 to 30 per cent of the cans were swollen; those receiving 10 mg. of sulphur as sodium thiosulphate did not show swelling of the cans in two years.

From these tests it is apparent that sulphur in almost any form will cause swelling of the cans and that sometimes very small quantities will suffice to produce this effect.

In 1926 the experiments were repeated, 100 mg. of sulphur per can being used in the forms employed in the preceding year, and in addition ferrous sulphide and sulphurous and sulphuric acid were included. These were canned both in cans that were exhausted and in unexhausted cans. The unexhausted cans containing sulphur swelled in a very short time, and even the unsprayed checks that were not exhausted developed a few flippers at the end of two years. The properly exhausted cans usually required several months longer to swell. According to MacInnes and Contieri (5), hydrogen overvoltage is increased when the gas pressure is decreased. Hence, properly exhausted cans liberate hydrogen with less ease than unexhausted cans. Sodium sulphide, ferrous sulphide, and sulphurous acid caused swelling of the cans, as did the other forms of sulphur. The sulphuric acid sometimes caused swelling, but frequently the cans pinholed before swelling.

It appears from these experiments that it is not hydrogen sulphide alone that causes the swelling. The gas present in the cans is largely hydrogen. The small quantity of hydrogen sulphide present accelerates the liberation of hydrogen caused by the action of the acids of the fruit on the metal of the container.

CORROSIVE ACTION OF SULPHUR ON THE CAN

In examining the lots of cans it was observed that the cans containing sulphur-sprayed fruit or having other forms of sulphur added to the fruit in the can appeared to be much more corroded than the check lots. It appeared that to a considerable extent the gas formation resulted from increased action of the fruit acids on the can.

To throw further light upon the mechanism of swelling and the corrosive action of sulphur upon the can, certain lots of cans were carefully weighed and filled with water or with 0.5 per cent citric acid. Citric acid in this concentration was used because it is the principal acid of the peach and because the total acidity is approximately of a concentration corresponding to 0.5 per cent citric acid. Various forms of sulphur were then added and the cans processed in the usual way, either with exhaust for three minutes or without any exhaust whatsoever. At the end of three months the cans were examined, one end was removed by a smooth cut, the cans were washed, dried, and again weighed. The loss in weight represents the extent of the corrosion that occurred. Much black sulphide

adhered to the cans receiving sulphur. This was removed by adding a little hydrochloric acid to the can for a few seconds and then quickly washing off the acid with water, drying, and weighing. Detailed notes were taken upon the appearance of the container, the odor of the contents, and the presence or absence of gas formation in the can. The results are shown in Table 1.

TABLE 1.—Corrosive action of various forms of sulphur upon the tin can

Substance added to the can	Treatment	Condition as to swelling	Odor of H ₂ S	Black discoloration	Perforation	Loss of weight per can (grams)
H ₂ O	3 minutes exhaust	Normal	Absent	Absent	Absent	0.011
0.5 per cent citric acid	do	do	do	do	do	.069
	No exhaust	do	do	do	do	.117
0.5 per cent citric acid and 50 mg. sulphur.	3 minutes exhaust	Swelled	Present	Present	do	.325
	No exhaust	do	do	do	do	.662
	3 minutes exhaust	do	do	do	do	.679
	No exhaust	do	do	do	do	.651
	Head space filled with nitrogen.	do	do	do	do	.898
0.5 per cent citric acid and 100 mg. sulphur.	Head space filled with hydrogen.	do	do	do	do	.838
	Head space filled with oxygen.	do	do	do	do	.737
Distilled water and 100 mg. sulphur.	3 minutes exhaust	Normal	Faint	do	do	.156
	No exhaust	do	do	do	do	.176
0.5 per cent citric acid and 100 mg. sulphur as hydrogen sulphide.	do	Swelled	Present	do	60 per cent.	.775
Distilled water and 100 mg. of sulphur as hydrogen sulphide.	do	Normal	Faint	do	Absent	.089
0.5 per cent citric acid and 400 mg. sodium hydrogen sulphite.	3 minutes exhaust	Swelled	do	do	do	1.161
	No exhaust	do	do	do	do	1.095
Distilled water and 400 mg. sodium hydrogen sulphite.	3 minutes exhaust	Normal	Absent	do	do	.237
	No exhaust	do	do	do	do	.270
0.5 per cent citric acid and 300 mg. sodium thiosulphate.	3 minutes exhaust	Swelled	Faint or absent.	do	do	1.201
	No exhaust	do	do	do	do	1.206
0.5 per cent citric acid and 5 c. c. of 5 per cent sulphurous acid.	3 minutes exhaust	do	do	do	20 per cent.	.974
	No exhaust	do	do	do	Absent	1.050
Distilled water and 5 c. c. of 5 per cent sulphurous acid.	3 minutes exhaust	Normal	Absent	do	do	.193
	No exhaust	do	do	do	do	.481
0.5 per cent citric acid and 500 mg. ferrous sulphide.	3 minutes exhaust	Swelled	Present	do	20 per cent.	1.327
	No exhaust	do	do	do	do	1.050
Distilled water and 500 mg. ferrous sulphide.	3 minutes exhaust	Normal	Absent or faint.	Absent	Absent	.020
	No exhaust	do	do	do	do	.025
0.5 per cent citric acid and 500 mg. stannous sulphide.	3 minutes exhaust	20 per cent flippers.	do	Present	do	.363
1 per cent citric acid and 500 mg. stannous sulphide.	do	do	Faint	do	40 per cent.	.674

It is evident from Table 1 that the properly exhausted cans containing citric acid were not greatly corroded but that the unexhausted cans were somewhat more severely attacked. The quantities of sulphur used caused much corrosion, and exhausting seemed to make comparatively little difference in the result. The same is true with cans in which the head space was filled with nitrogen, hydrogen, or oxygen. In most cases where sulphur had been added at least a faint odor of hydrogen sulphide was present. It was always more pronounced in cans containing acid and was faint or absent in cans filled with sulphur and distilled water. Perforations occurred in several lots, and undoubtedly many more would have developed had the experiment been continued longer.

It is noted that no swelling occurred where distilled water and sulphur were canned together, whereas the cans filled with 0.5 per

cent citric acid with sulphur added invariably swelled. It is apparent that the acid of the fruit is the primary cause of the formation of the gas. The sulphur seems merely to accelerate a tendency already present. The cans containing sulphur in such forms that it could combine with the metal of the container, however, were corroded regardless of the presence of the acid. For example, hydrogen sulphide corroded the can whether acid was present or not, but it did not swell the can unless acid was present. Ferrous sulphide in the presence of acid severely corrodes and causes swelling of the can, but when it is canned with water no corrosion and no gas formation occurs.

These experiments indicate that the spray residue reacts upon the metal of the container to form sulphides. If acid is present the metallic sulphides are decomposed and hydrogen sulphide is formed, at least to a small extent. The hydrogen sulphide thus liberated accelerates the liberation of hydrogen gas as a result of the action of the acid upon the metal of the container.

LIBERATION OF GAS FROM IRON AND TIN

To determine whether the formation of the hydrogen gas was the result of the action of the acids upon the tin or the iron of the can, strips of steel plate, such as are used in the manufacture of cans, and plates of block tin of equal size were immersed in citric acid or water in Ehrlenmeyer flasks, with ordinary burettes so attached that any gas formed could easily be detected and measured. In another set of flasks with iron and tin immersed in citric acid the changes in pressure in the flasks were measured by means of manometers. From the changes in pressure the quantity of gas formed could be computed. The two methods agreed in their general result. A number of these tests were set up with the addition of various forms of sulphur, and in no case were appreciable quantities of gas liberated from tin. This is probably due to the high overvoltage of hydrogen on tin, as shown by Newbery (7). If oxygen was present the pressure in the flasks was lowered, indicating the absorption of that element. No appreciable quantity of hydrogen was liberated from iron in distilled water, but hydrogen was liberated from iron immersed in 0.5 per cent citric acid whether sulphur was present or not. Liberation of hydrogen was always much more rapid in the presence of sulphur.

The foregoing experiments show that citric acid of the concentration present in the peach will cause hydrogen-gas formation when in contact with iron alone.

ELECTROCHEMICAL NATURE OF THE CORROSIVE PROCESS

Since the iron of the tin can is more or less imperfectly covered with tin, it seems desirable to know why the fruit rarely causes swelling in the absence of sulphur although there is corrosion and gas formation when small quantities of sulphur are present. This seems to find rather adequate explanation in the electrochemical theory of corrosion (2, 8, 11).

To discover what effect the placing of the metals iron and tin in contact would have upon the nature and extent of corrosion when immersed in peach juice, an experiment was carried out wherein 700 c. c. of juice from the Belle variety was placed in each of four

ordinary fruit jars. In the first jar a piece of iron 3 by 4 inches (7.5 by 10 cm.) such as is used in the manufacture of tin cans was placed; in the second, a piece of block tin of equal size was placed; in the third, a piece of iron and a piece of tin were placed but not in contact; in the fourth, pieces of iron and tin firmly in contact were placed. The jars were then put into a retort and steamed for 20 minutes, after which the clips were snapped down, sealing the jar. By this method most of the oxygen was excluded. Before the pieces of tin and iron were introduced into the jars, the metals were carefully cleaned with hydrochloric acid, dried, and weighed.

At the end of six months the experiments were terminated, and the strips of iron and tin were washed, dried, and weighed again. In the first jar the iron (alone) lost 0.555 gm.; the tin (alone) in the second jar lost 0.009 gm. In the third jar with the two metals not in contact the iron lost 0.167 gm. and the tin lost 0.164 gm.; in the fourth jar with the metals in contact the iron lost 0.038 gm. and the tin lost 0.212 gm. It is evident that the iron is greatly protected from corrosion by contact with tin, and that tin in contact with iron is subjected to much greater corrosion. These results are in general agreement with the findings of Kohman (3) and also with those of Lueck and Blair (4). It is noted that the total corrosion occurring in the fourth jar is somewhat less than the total in the third jar and much less than in the first jar, with iron alone. It appears that the iron is protected to some extent when tin is present in the same vessel, even when they are not in contact. These tests would indicate that tin is anodic to iron when they are used as electrodes in a galvanic cell with peach juice as the electrolyte. The tin behaves as the dissolving electrode in such a cell. This would indicate that in ordinary canning operations iron is generally cathodic to tin.

A cell containing peach juice as electrolyte and iron and tin as electrodes was set up. The resulting potential was small, but consistently indicated that iron was cathodic to tin. If there were ferric ions in the electrolyte this result is just what would be expected from their position in the electrochemical series. In accordance with the normal electrode potentials (10), divalent iron is above tin and trivalent iron below tin in the electrochemical series. Since oxygen was present to some extent in the cells heretofore described, it seems probable that enough ferric iron was present to give the polarity indicated.

EFFECT OF OXYGEN AND SULPHUR ON POLARITY OF IRON AND TIN

To show the effect of oxygen and sulphur on the polarity of iron and tin, the cells indicated in Table 2 were set up.

The electrode vessels used were wide-mouthed jars fitted with rubber stoppers. These were filled about three-fourths full of citric acid (700 c. c.) as electrolyte. The acid was boiled just previous to being placed in the jars. Through the rubber stoppers a 0.5 per cent citric acid-agar bridge connecting the vessels containing the electrodes was inserted. The electrodes were carefully cleaned with hydrochloric acid, washed with distilled water, wiped with a clean dry towel, and immediately dried for a short time in an oven. They were then inserted through the rubber stoppers. Glass stopcocks were also inserted through the stoppers to pass nitrogen or oxygen

through the electrode vessels as desired. Where the presence of sulphur is indicated, 0.3 gm. of flowers of sulphur was added to the electrode vessel. A little hydrogen sulphide was also added at the time the electrodes were inserted. The cells thus set up were kept in a room with a constant temperature of 30° C.

Diagram indicating the polarity of iron and tin under varying conditions

No.	Diagram of cell				Resulting polarity
1	Iron...	Citric acid, oxygen excluded.	0.5 per cent citric acid, agar-agar bridge.	0.5 per cent citric acid, oxygen excluded.	Tin... Iron anodic at first, but became cathodic.
2	Iron	0.5 per cent citric acid, oxygen present.	0.5 per cent citric acid, agar-agar bridge.	0.5 per cent citric acid, oxygen excluded.	Tin... Iron cathodic.
3	Iron	0.5 per cent citric acid, oxygen excluded.	0.5 per cent citric acid, agar-agar bridge.	0.5 per cent citric acid, oxygen present.	Tin... Iron anodic.
4	Iron...	0.5 per cent citric acid, sulphur present, oxygen excluded.	0.5 per cent citric acid, agar-agar bridge.	0.5 per cent citric acid, sulphur present, oxygen absent.	Tin... Iron anodic.
5	Iron	0.5 per cent citric acid, sulphur present, oxygen absent.	0.5 per cent citric acid, agar-agar bridge.	0.5 per cent citric acid, sulphur absent, oxygen absent.	Tin... Iron anodic.
6	Iron	0.5 per cent citric acid, sulphur absent, oxygen present.	0.5 per cent citric acid, agar-agar bridge.	0.5 per cent citric acid, sulphur present, oxygen absent.	Tin... Iron cathodic.

The potential between tin and iron was never very great, and the polarity could frequently be changed by bubbling the gas through one electrode vessel and not through the other. The potential difference was measured at frequent intervals for 20 days. The potential was variable and drifted one way or the other depending upon the rate at which oxygen or nitrogen was bubbled through the vessels. The polarity was quite generally that indicated in Table 2. Since the condition of the surface of the electrodes is so very important, the results obtained here should be considered only as indicating a general tendency. It seems that where oxygen is present the tendency is for iron to be cathodic and where hydrogen sulphide is present the general tendency is for iron to be anodic. This is in general agreement with the normal electrode potentials of tin and of divalent and trivalent iron (10). Divalent iron stands above tin, whereas trivalent iron stands below tin in the electrochemical series. The experiments indicate that in ordinary canning operations tin is anodic to iron, but where hydrogen sulphide is present the ferric salts are reduced to the ferrous form, and iron may become anodic to tin. Oxygen also acts to prevent the formation of a polarizing film of hydrogen. The spray residue therefore tends to reverse the polarity between tin and iron and makes the iron the dissolving electrode of a galvanic cell wherever the tin does not completely cover the iron.

The galvanic action between iron and tin does not account for the increased liberation of gas when sulphur is present. When iron is anodic and is being dissolved by an acid the hydrogen must be liberated at the cathode. Since in these experiments tin has never been observed to liberate hydrogen, it would appear that the hydrogen is liberated from the iron directly as a result of localized chemical action upon the iron. When free sulphur was present the liberation of gas from the particles of sulphur has been observed. In this case the electrochemical action was between iron as anode and sulphur as cathode.

The corroding action of an electrolyte is generally slowed down by the accumulation of ions of the corroding metal, and the corrosion process may even be stopped altogether. In this case hydrogen sulphide prevents the accumulation of ferrous and ferric ions and therefore maintains the tendency to dissolve at its maximum value. This must increase the tendency to liberate hydrogen or hydrogen sulphide and also tends to make iron anodic to tin.

POLARITY OF IRON AND TIN AS RELATED TO CORROSION

To determine just how tin and iron react in an electrolyte when sulphur is present, an experiment was performed wherein 700 c. c. of 0.5 per cent citric acid was placed in each of four ordinary fruit jars. The acid was boiled just previous to being placed in the jars. To each jar 0.3 gm. of flowers of sulphur was added. Plates of iron and tin were introduced into these jars as follows: In the first, iron alone; in the second, tin alone; in the third, tin and iron not in contact; and in the fourth, iron and tin placed firmly in contact. The jars were then heated 20 minutes in a steam bath, after which the tops were clamped down and the jars sealed as in ordinary canning operations. They were thus very nearly free from oxygen. After the jars had been stored for 134 days the metal plates were removed, dipped for an instant in hydrochloric acid, then washed with water, wiped with a dry towel, dried for a short time in an oven, and weighed.

In the first jar the iron (alone) lost 1.126 gm.; in the second the tin (alone) lost 0.172 gm. In the third jar, with tin and iron not in contact, the tin lost 0.12 gm. and the iron lost 1.673 gm.; in the fourth, with tin and iron placed firmly in contact, the tin lost 0.013 gm., and the iron lost 2.198 gm.

It is therefore evident that the presence of sulphur causes intense corrosion of the iron. The tin corrodes very much less than the iron. The tin in contact with the iron does not protect it, but on the other hand the tin is protected to some extent from corrosion by contact with iron.

This is the exact reverse of the relationship obtained with the same metals when immersed in peach juice and in citric acid without the addition of sulphur. This seems satisfactorily explained by the relative positions of tin and of divalent and trivalent iron in the electrochemical series. With an oxidizing acid and with more or less oxygen present, as in the peach juice, ferric iron may be present, which would make the tin the dissolving electrode. If hydrogen sulphide is present any ferric iron would be reduced to ferrous iron, and therefore the iron would be the dissolving electrode, or anodic to tin when in contact with it. It seems that where oxygen or an oxi-

dizing acid is present a certain degree of passivity is conferred upon the iron, and the addition of hydrogen sulphide destroys this passivity. The effect of oxygen and oxygen-supplying acids may also partly explain some of the results of Mantell and Lincoln (6), of Lueck and Blair (4), and of Kohman (3). Of course many factors are concerned, such as temperature, nature of the electrolyte, concentration of the metallic ions in the electrolyte, the character of the surfaces of the corroding metals, and their overvoltage values. From these experiments, however, it is clear that sulphur is of considerable importance in canning operations, since many vegetables are known to liberate hydrogen sulphide during sterilization. Even immature fruits may liberate small quantities of hydrogen sulphide when heated. Much more work will be required to determine the exact effect of sulphur with different products and to control its action favorably in the canning process.

SUMMARY

Sulphur-spray residues are sometimes rather difficult to remove from the surface of peaches. The presence of this sulphur in the can causes the formation of hydrogen sulphide and hydrogen gas, with the consequent swelling of the can.

Washing in cold water and even in hot water does not always prove effective in removing the spray material. Dipping the fruit in hot dilute lye for a few seconds and then washing the fruit with jets of cold water has proved effective in the two seasons in which it has been used. It has been found possible in this way to handle rather soft fruit without disintegrating it.

Almost all forms of sulphur in the acid juices of the peach cause the swelling of the can. Lime-sulphur spray, hydrogen sulphide, sodium sulphide, sodium sulphite, sodium thiosulphate, flowers of sulphur, ferrous sulphide, stannous sulphide, and sulphurous acid have caused swelling of tin cans in experimental packs.

Aside from causing the can to swell sulphur has a corroding effect upon it. In addition to itself combining with the metal, it seems to act as a catalytic agent, greatly accelerating the action of the natural fruit acids upon the metal of the container.

In the presence of organic acids most forms of sulphur will cause corrosion and swelling of the can, but in the absence of these no swelling of the can occurs. However, if the sulphur is in such form that it can combine with the metal it will corrode the can in the absence of acid. Thus, flowers of sulphur corrodes the can whether acid is present or not; ferrous sulphide does not cause appreciable corrosion in absence of acid, but in the presence of acid it greatly corrodes the can.

The action of organic acids upon tin does not result in the liberation of hydrogen, but hydrogen is liberated from iron by organic acids. Thus it seems that if the iron in the container could be completely covered with tin the possibility of gas formation would be much less.

The electrochemical theory of corrosion seems to offer a rather adequate explanation of the phenomena here observed.

In ordinary canning the acid juices of the peach act as an electrolyte in which iron is cathodic to tin when they are in contact. If hydrogen sulphide is present this relationship is reversed, and iron becomes anodic and tin cathodic.

In the presence of oxygen or an oxidizing acid, iron is usually cathodic to tin, whereas if a reducing agent such as hydrogen sulphide is present, the iron is anodic to tin when they are in contact. This relationship, together with the fact that hydrogen sulphide accelerates the liberation of hydrogen from iron, seems to account completely for the swelling of the cans in the cases here described.

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STUDIES ON *SCLEROTIUM ROLFSSII*, WITH SPECIAL REFERENCE TO THE METABOLIC INTERCHANGE BETWEEN SOIL INHABITANTS¹

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INTRODUCTION

During the past decade soil-inhabiting plant pathogenes have received an increasing share of attention from plant pathologists, and a considerable amount of information has been accumulated concerning the importance of various climatic and soil factors, soil temperatures in particular, in the inception and development of disease (9, 10).²

While various investigators had previously called attention to the importance of environmental factors in influencing disease expression of various pathogenes, the relative newness of these studies may be judged by the fact that, while such a factor as temperature was substantially recognized as a conditioning agent by Pasteur and his associates in 1878 (16) in their study of the immunity and susceptibility of chickens to anthrax, very little attention had been given to such studies, at least by plant pathologists, until the past few years.

It is, therefore, not surprising that a number of phenomena relative to environmental conditions remain to be investigated, and that some results obtained by varying only one or two environmental factors will be materially modified when a number of natural environmental conditions acting simultaneously are carefully studied under controlled conditions. In this article attention will be directed to the interaction of two soil fungi in limiting or enhancing each other's growth. The studies here reported involve principally *Sclerotium rolfssii*, a fungus which seems to be world-wide in its distribution.

Numerous citations may be found in mycological and pathological literature to the interaction of various fungi, bacteria, and protozoa, but as these have been well summarized by Harder (5) and more recently by Porter (18) there is no need of considering them here. Many of these references concern the inhibitory or stimulatory action of a contaminant obtained in the process of culturing some pathogene. Others refer to the production of fruiting bodies when two strains of some *Mucor*, *Glomerella*, or other species of fungi are brought together. Very little is to be found on the influence of inhabitants of the soil in limiting or stimulating growth and parasitism of soil-inhabiting and root-invading plant pathogenes. Porter was concerned largely with the modifications in growth brought about in a large and heterogeneous collection of fungi and bacteria when two or more are brought together. Incidental to this he found that a bacterial species, designated as No. 45, strongly inhibited the growth on culture media of various fungi, including pathogenic species of *Helminthosporium* and

¹ Received for publication Feb. 6, 1929; issued July, 1929. Research paper No. 131, Journal Series, University of Arkansas.

² Reference is made by number (italic) to "Literature cited," p. 60.

the flax-wilt fungus, *Fusarium lini*. He also found some inhibitory effect on *Helminthosporium* when seedlings of wheat were grown on soil inoculated with No. 45 as well as with *Helminthosporium*. On the other hand, he obtained negative results when he attempted to inhibit the pathogenicity of *F. lini* on flax seedlings by using the same bacterium. Porter does not indicate the natural habitat of his No. 45 and notes that it grows so slowly on soil "that it is doubtful whether it would be very useful in checking the normal soil flora."

THE 1928 EPIDEMIC OF CANTALOUPE ROT

One of the reasons which led the writers to use *Sclerotium rolfsii* in these studies was the extraordinary destructiveness of this fungus during the season of 1928. The disease was first noticed about the middle of July in the region around Hope, Hempstead County, Ark., one of the most important cantaloupe centers in the South. It appeared as a soft, mushy rot of both mature and immature fruit. Frequently the rot could be seen on cantaloupes which were not more than one-third grown, and in such cases the fruit failed to mature and quickly rotted. The most noticeable cases, however, appeared on fruits that were reaching maturity. In such instances, when the fruit was about to be severed from the plant, a whitish, moldy growth could be seen on the sides, and as the lower surface was exposed the mold would frequently be found in greater abundance than on the sides, indicating that the portion of the fruit lying next to the soil served as the primary infection center. Very often this region was more or less rotted, the skin being disrupted, and a sweetish, fermented liquid oozing out of the flesh. If close attention were paid to the soil around the rotted fruit the same whitish mold would be seen extending in fan-shaped fashion for some distance from the attacked plant, growing on the soil, during periods of moist weather. In dry weather it would not be noticed on the soil or to any great extent on the fruit.

While the disease here involved is a southern malady of a large number of different plants (28 new hosts (15) having been added recently), including potatoes, sweet potatoes, tomatoes, watermelons, cabbages, and beans, it apparently did not cause any serious losses to these crops in 1928.

The same disease was reported in epidemic form in the eastern cantaloupe-growing region, around Norfolk, Va.,³ about a week after it was identified in Arkansas. There also it appeared to be confined to the cantaloupe fruit and was unnoticed on eggplants in spite of the fact that it had been observed on the latter crop during the seasons of 1926 and 1927. In Arkansas it was found not only around Hope but in other localities where cantaloupes are grown extensively. It thus appears reasonable to suppose that either a particular, specialized strain of the parasite became active in 1928 or that environmental conditions in diverse parts of the country were such as to render the cantaloupe fruit particularly vulnerable. The latter explanation seems to be more tenable, inasmuch as pure-culture isolations from cantaloupes have given the writers successful infections on young tomato plants, with the production of typical stem rot.

³ UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. SCLEROTIUM ROT OF CANTALOUPE. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 12: 64-65. 1928. [Mimeographed.]

While the southern rot has for many years been known as a storage as well as a field disease, it has rarely been found to act exclusively as a disease of the fruit. In the field it usually attacks the young stems, close to the soil line, causing primarily a rot of the cortex although it may also involve the woody cylinder. Sometimes it has been found attacking older stems and producing similar symptoms. Evidently conditions governing stem infections precluded such attacks in 1928.



FIGURE 1.—Cantaloupes that had been in transit for 24 hours attacked by *Sclerotium rolfsii*. Note the severe rot induced by this fungus on A and the mycelial development on the newspaper wrappers around B

The nature of these limiting conditions remains unknown. There is, of course, the possibility that some stem rot did occur but went unnoticed or was attributed to some other cause.

Another factor which makes the recent epidemic of special interest is that the malady would often go unnoticed on the fruit when it was first crated, but when it arrived at the terminal markets the inspector reported considerable "Sclerotium rot." On fruit in transit the fungus has been known to grow with such rapidity as more or less to cover the wrapping papers that happened to be in contact with the fruit. Within 24 hours after shipment it has been found spreading over sheets of newspaper that had been wrapped around the cantaloupes. (Fig. 1, B.) This fungus can very readily utilize

cellulose for nutrient purposes, as was determined by growing the fungus on moistened, sterilized filter paper. It produced a noticeable, though not heavy, mycelial growth as well as sclerotia on this medium.

From the knowledge now at hand one may hazard a guess as to the reason for the extreme prevalence of this disease in 1928. The disease producer has in the past been known to be widely distributed in the United States over considerable areas south of the Mason-Dixon line, and has at times been reported from more northern regions

(15). But in spite of this wide distribution its extreme destructiveness has been more or less limited to certain localities situated in the extreme southern parts of the United States. While it probably was present in the Arkansas cantaloupe fields prior to 1928 it caused insufficient damage to attract attention. In 1927, for example, cantaloupes are not mentioned in the plant-disease survey⁴ as hosts for *Sclerotium rolfsii* in Arkansas, although sweet-potato slips and tomato plants are noted as having been attacked. It will be recalled that the summers of 1927 and 1928 were exceptionally moist and rainy over a large part of the country east of the Rocky Mountains, particularly during the earlier part of the southern cantaloupe-growing periods. The United States Weather Bureau reports for 1928 the wettest June on record for Arkansas and also gives positive departures in rainfall during the first half of July for the Hope cantaloupe region. During 1927 with its tremendous rainfall and destructive floods over wide areas, particularly in the Mississippi Valley region, it may be surmised that the parasite had exceptionally favorable conditions for its development. Various investigators (1, 17, 20, 25) have in the past reported the marked proclivities of this fungus for moist conditions, and when 1928 in part duplicated these conditions the parasite ran wild.

GROWTH OF *SCLEROTIUM ROLFSII* ON POTATO-DEXTROSE AGAR VARYING IN H-ION CONCENTRATION

In his studies on *Sclerotium rolfsii* Higgins (8) has brought to light some interesting facts concerning the relationship of the growth of this parasite to variations in H-ion concentration. He found this fungus to be markedly inhibited in alkaline media and unaffected or even stimulated in growth in rather strongly acid ranges of H-ion concentration. The inhibitory effect of the former was so marked that it is not surprising to find him recommending in an earlier paper (7) the use of lime on infested fields for the control of this fungus and of the disease which it produces. It is quite obvious, however, that a serious objection may be raised to this work, one that Higgins himself was doubtless cognizant of, namely, that he used beef-extract broth for the test medium. This was chosen because it was found to be better adapted for the growth of the fungus than solutions containing mineral nutrients, and because the different levels of pH values could be determined much more readily than on other media by the methods used.

It, therefore, seemed worth while to duplicate Higgins's work, but instead of using materials like beef extract or commercial peptone, substances which are apt to be quite foreign to a soil-inhabiting plant pathogene, it was decided to use a vegetable decoction which in previous tests had promoted excellent growth of the fungus. The medium used was common potato-extract agar to which sufficient dextrose was added to make a 2 per cent solution. It is realized that this is also quite unnatural, but for artificial media a vegetable decoction more nearly approaches certain soil ingredients than do animal products of the type mentioned above. By checking the pH values

⁴ McMILLAN, H. G. DISEASES OF VEGETABLES AND FIELD CROPS (OTHER THAN CEREALS) IN THE UNITED STATES IN 1927. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 61, 300 p. 1928. [Mimeographed.]

obtained by the ordinary comparator block method against the pH values obtained by using Wherry's double-wedge comparator it was possible to measure the H-ion concentration with an error of less than 0.1 of a pH division. It should be noted that the buffer index of potato extract is relatively high so that the acidity or alkalinity of the color indicators would not be expected in this case to interfere with colorimetric determinations. The turbidity of the medium was not sufficient to prevent accurate comparisons with known standards, and in every instance a tube or capsule was used, depending on the method, containing the unknown without indicator to mask the color of the known standard.

In the first series of tests the basic medium was made according to the following formula: 500 gm. peeled potatoes, 20 gm. dextrose, 20 gm. agar-agar, 1,000 c. c. of distilled water. The cut potatoes were boiled until the pieces were almost ready to fall apart, the fluid filtered several times through closely woven cloth, the other ingredients incorporated, and sufficient water added to make up the desired amount. The medium was divided into 100 c. c. quantities and autoclaved at 15 pounds pressure for about 15 minutes. After sterilization, the required amounts of N/1 NaOH and N/1 HCl were added to each batch so as to give the following series of H-ion concentrations: pH 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, and 9. The acid and alkaline solutions were added aseptically in a small transfer chamber which had been thoroughly steamed. The H-ion concentrations were determined in the manner previously indicated and precautions taken in each case to see that the final pH value of each solution corresponded with the one desired. The tests were conducted in triplicate form, three plates of approximately 10 c. c. of medium being poured for each pH. Finally the Petri dishes were incubated for 24 hours prior to inoculation so as to be sure that contaminations had not occurred.⁵

Higgins (8) had found that an equal quantity of inoculum could be obtained with relative ease by using fully developed sclerotia that had been freed from the mycelium. The present writers have determined that while these bodies vary in size, nevertheless their rates of growth in most instances are comparable at a given H-ion concentration on the same nutrient medium. However, it seemed desirable to have a further check on this, so in several instances two sclerotia on one plate were introduced in order to determine the relative quantity of growth that may be expected in a given length of time from different quantities of inoculum. In this manner it was determined that variations in growth at one pH level occur rather frequently even with sclerotia of approximately equal size, particularly during the first 48 hours. But as the period of incubation was increased up to 72 hours the colonies of any one series tended to become equal, and the variations which occurred in no way interfered with the detection of a very marked difference in growth observed at the different levels. All the plates were kept in an incubator maintained at a temperature of about 29° C.

The colonies were carefully measured and notes were taken for a number of days after the inoculations. Forty hours after the introduction of the sclerotia, growth had occurred in all the plates except

⁵ The writers are indebted to A. L. Smith for assistance in these initial tests.

one at pH 3, one at pH 7.5, and all at pH 8, 8.5, and 9. At the end of 72 hours all the sclerotia had germinated up to pH 8. From that point upward to pH 9 no growth could be detected. Table 1 shows the amount of growth obtained at the various H-ion concentrations.

TABLE 1.—*Growth of Sclerotium rolsii at different H-ion concentrations on a potato-dextrose agar medium*

H-ion concentration (pH)	Average diameter in centimeters of colonies at end of 72 hours	Notes taken at end of 120 hours
3.....	2.3.....	All plates full of mycelium except one; no sclerotia.
3.5.....	3.8.....	All plates full of mycelium; few sclerotia.
4.....	3.08.....	All plates full of mycelium; more sclerotia than at pH 3.5.
4.5.....	3.8.....	All plates full of mycelium; sclerotia more abundant than at pH 4.
5.....	3.75.....	All plates full of mycelium; sclerotia more abundant than at pH 4.5.
5.5.....	3.3.....	All plates full of mycelium; same number of sclerotia as at pH 5.
6.....	3.5.....	All plates full of mycelium; greatest number of sclerotia.
6.5.....	3.7.....	All plates full of mycelium; not as many sclerotia as at pH 6.
7.....	1.5.....	Mycelial growth less than at pH 6.5; sclerotia fewer in number.
7.5.....	1.41.....	Growth of mycelium more restricted than at pH 7; few sclerotia.
8.....	No growth.....	No growth.
8.5.....	do.....	Do.
9.....	do.....	Do.

Table 1 shows that between the H-ion concentrations of pH 3.5 and 6.5 there are but minor differences in growth of mycelium. The writers believe that these differences are entirely fortuitous and of little significance. There seems to be, however, a noticeable gradient in sclerotial development and if these bodies are to be regarded as part of a vegetative thallus and are not to be compared with true fruiting bodies then it can be said that there is a noticeable difference in vegetative growth between these levels of H-ion concentration. This interpretation would seemingly be all the more proper because of Higgins's (6) morphological and cytological studies. He found the mycelium to be binucleate and made up of both fine and coarse threads. Clamp connections in the broad threads were commonly observed. The present writers have noted the same thing. Higgins also noted that the finer types of hyphae enter into the formation of sclerotia, which "first appear as small white tufts of loosely intertwined small branches." He found that the cells within the center of the mass eventually became vacuolate and usually multinucleate. The most important of these findings, for the present purposes, are the binucleate mycelium, the clamp connections—these being the characters which with obvious propriety led Higgins to believe that this fungus may be classed with the Basidiomycetes—and the coenocytic nature of some of the cells. These characters as well as the parallel arrangement of hyphae, a phenomenon which the present writers have found to be of common occurrence in this fungus, all suggest the diploid, secondary mycelium of higher Basidiomycetes.

Table 1 shows a noticeable falling off in growth of mycelium, as well as in sclerotial development, when the neutral point, pH 7, is reached. This was particularly evident at the end of 72 hours and less marked as the period of incubation became longer. The explanation of this becomes evident as one notes that beginning with pH 7 and extending into the alkaline range there is a very noticeable

negative departure in growth, ceasing altogether when pH 8 is reached. The indications are clear that given the type and quantity of inoculum contained in a single sclerotium this fungus is markedly inhibited by hydroxyl ions, and that if these are not sufficiently powerful to inhibit growth completely the fungus will in time overcome the initial inhibitory effect by its marked ability to produce acid. The writers have found that within 72 hours after inoculation oxalate crystals could be readily detected in mounts taken from any plate in which growth had occurred. This is in accord with Higgins's results (8) in which he found the reaction of alkaline broth to have changed to a distinctly acid one. For example, he notes that broth testing pH 7.5 will after several weeks' growth of the fungus test pH 4 to pH 4.2. Likewise he called attention to the marked ability of this fungus to produce oxalic acid on various media; in fact, he considered the killing of host tissue by this fungus as being due to this acid. The writers therefore feel justified in suggesting that on media of relatively low alkalinity the initial lag in growth will in time disappear because the metabolic products produced by the fungus enables it to overcome the harmful action of the alkaline medium, and this would tend eventually to equalize the growth at various H-ion concentrations. The writers are not suggesting that staling substances produced by the fungus are absent from the media upon which *Sclerotium rolfsii* has been growing, but if they are formed they play little or no rôle in inhibiting growth of this fungus for some time at least after inoculation, for the fungus permeates not only the bottom of a Petri dish but also the sides and top and often protrudes for some distance around the edges, all within the course of about a week. Its exceptional rapidity of growth is to be observed not only in artificial culture but also under natural conditions.

The final and perhaps the most important point shown in Table 1 is the total absence of growth on potato-dextrose agar at pH 8 and above. This is in agreement with Higgins's results on beef-extract broth in which three of the strains utilized failed to grow beyond pH 8.1 and only one strain grew at pH 8.3. It thus appears that comparable results on diverse media may be expected with reference to the limiting action of OH ions on the germination and growth of sclerotia of *Sclerotium rolfsii*. If such limitation could be obtained in soil which is infested with this fungus in the sclerotial stage, then it is quite obvious that a ready available control measure is at hand, and in accordance with this Higgins (7) in an earlier paper expressed the belief that the application of lime, 1 ton stone or 1.5 tons hydrated lime to the acre, would prove beneficial. This is not the first time that liming has been suggested as a possible control for this fungus. In 1908 Fulton (3, p. 7) found that *S. rolfsii* will not grow on nutrient media unless they have a high degree of acidity.

This suggests that the fungus may find conditions best for its growth only in soils that are more than ordinarily acid. If this is really the case, the use of lime to reduce the acidity of the soil ought to have a good effect in reducing the loss from blight.

As far as the writers know, Nakata (13) is the only investigator who has not found this fungus to be adversely influenced by alkalinity. However, as his English summary does not present the details of his work and as his Japanese text is unintelligible to the present writers, it is quite possible that this seeming discrepancy can be explained as

being due to other factors. It will be shown later on that the kind of substratum as well as the quantity and type of inoculum can materially modify the harmful influence of alkalinity. In his later work (8), however, Higgins gave up the idea that liming could be relied upon to give satisfactory control because he found it very difficult to maintain a soil reaction above pH 8 throughout a single season. Indeed, even with the use of 5 tons of hydrated lime per acre, he found the soil reaction to be barely above pH 7 and the attacks of the fungus were not inhibited.

A serious objection may be raised to the tests which have just been described, namely, the obvious impropriety of using pure cultures of a soil-inhabiting fungus on media which are free from other microorganisms. Under natural field conditions such relationships do not exist. The fact that plant pathologists as a whole have paid very little attention to the necessity of studying the natural companions of any particular microorganism does not in the least obviate the desirability of such studies. The senior writer has already called attention to this (23), and the recent studies of Millard and Taylor (11) on the relationship of nonpathogenic strains of *Actinomyces* in limiting infections of pathogenic strains in the same genus add emphasis to the necessity of such studies.

With this in mind the writers attempted in a preliminary way to determine the response of *Sclerotium rolfsii* to the presence of another soil inhabitant commonly present in the cotton areas of the South, namely, *Fusarium vasinfectum*. (It should be noted that commercial cantaloupe fields in Arkansas are almost entirely within the cotton-growing region of the State.) At the same time it was deemed desirable to check any possible growth-limiting factor, aside from the preponderance of OH ions, which may have prevented the germination of the sclerotia of *S. rolfsii* on those media which tested pH 8 and above. Accordingly those plates which had shown no growth in the alkaline ranges were inoculated with the cotton-wilt pathogene and in every instance growth of this fungus was encompassed. This is in full accord with Neal's work (14) on *F. vasinfectum* in which he found this fungus capable of growing on alkaline media testing as high as pH 9. It was thus further ascertained that the concentration of OH ions was probably responsible for the lack of germination of the sclerotia.

One other factor was taken into consideration, namely, the ability of numerous organisms to change the reaction of a substratum during the process of growth. It is now firmly established that an acid medium may change toward the neutral point and, conversely, that an alkaline medium may be rendered neutral or even acid in the presence of a growing organism. In a former article the senior writer (22) reported the cotton-wilt pathogene as changing the reaction of a medium from pH 4.2 to pH 6.6. Neal (14) obtained similar results and further determined the ability of *Fusarium vasinfectum* to change an alkaline medium toward the neutral point. If these findings are correct, then, unless some other factor intervenes, it should be possible to obtain growth of *Sclerotium rolfsii* on media originally alkaline which, in the process of sustaining growth of some other microorganism, has lost its alkaline properties.

In order to test this theory all plates, originally testing pH 8 to pH 9, which had failed to produce growth of *Sclerotium rolfsii*, and

which had permitted the growth of *Fusarium vasinfectum* were, after 48 hours' incubation of the latter fungus, reinoculated with mycelium of *S. rolfsii*. It should be noted that the original sclerotial inoculum failed to grow and that the cotton-wilt fungus had made considerable growth within the period given. Tests with Clark and Lub's (2) indicators clearly showed a change in reaction of the medium around the *Fusarium* colonies. With the introduction of fresh inoculum abundant growth of *S. rolfsii* took place in every case and it was therefore concluded that the change in reaction of the medium was primarily responsible for the reversal in growth relationship of this fungus. The fact that the initial inoculum in the form of sclerotia had failed to respond to the changed conditions was attributed to the overcrowding of the *Fusarium* colonies on the sclerotia. It will be shown later that this is not entirely a safe deduction and that the difference in types of medium as well as in quantity of inoculum has a marked influence on growth reactions, irrespective of the OH-ion concentration.

GROWTH OF *SCLEROTIUM ROLFSII* ON DEHYDRATED POTATO-DEXTROSE AGAR VARYING IN H-ION CONCENTRATION

One of the reasons which originally led the writers to use potato-dextrose agar instead of the beef-broth medium used by Higgins was the difference in growth that one may expect with media of different chemical composition. Thus Rosen (21, p. 365) in 1922 showed that at the same H-ion concentration some media may sustain growth while others inhibit it. In view of this fact and because potato-dextrose agar made in the laboratory may be expected to vary, it appeared worth while to check the results previously given by using a more or less standardized medium of similar chemical composition. Use was made of dehydrated potato-dextrose agar manufactured by a well-known commercial concern which specializes in such products. The same type of inoculum was used and the determinations of the various H-ion concentrations were conducted in the same way as those mentioned previously. Table 2 presents in brief the results of these tests, and Figures 2 and 3 show the nature of the growths produced at different H-ion concentrations.

TABLE 2.—Growth of *Sclerotium rolfsii* at different H-ion concentrations on dehydrated potato-dextrose agar

H-ion concentration (pH)	Average diameter in centimeters of colonies at end of 72 hours	Notes taken at end of 120 hours
3.....	3.6.....	All plates full of mycelium.
4.....	4.....	Do.
5.....	4.2.....	Do.
5.5.....	4.....	Do.
6.....	4.6.....	Do.
6.5.....	4.7.....	Do.
6.9.....	2.....	One plate full of mycelium, others almost full.
7.5.....	0.7.....	Mycelial growth markedly restricted, plates about two-thirds filled.
8.....	Faint growth.....	Average diameter of colonies, 2 cm.
8.2.....	No growth.....	Average diameter of colonies, 1.5 cm.
8.5.....	do.....	Average diameter of colonies, 1.2 cm.
9.....	do.....	Slight growth in all plates.

An inspection of Table 2 reveals the marked superiority of the dehydrated medium over the ordinary potato-dextrose agar in promoting the growth of this fungus in practically the whole range of H-ion concentration. Although the same general sort of relationship to acidity and alkalinity can be observed on this medium (figs. 2 and 3) as in the one previously given, it will be seen that at pH 8 and above, unlike the former, growth at the end of 120 hours is not entirely inhibited. Why this difference should exist in media of

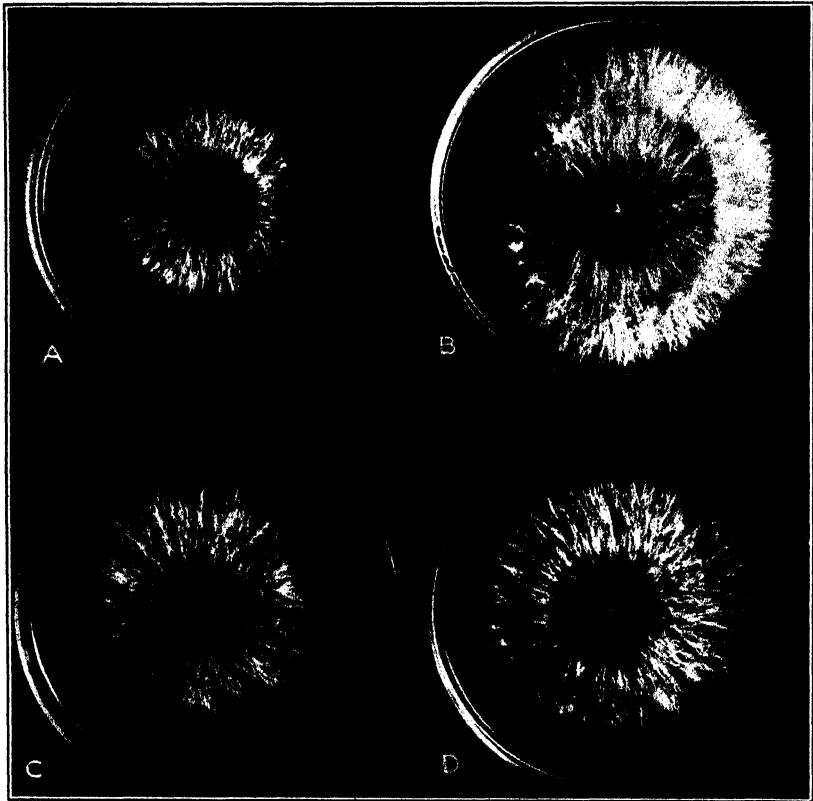


FIGURE 2.—Ninety-six-hour growth of *Sclerotium rolfsii* on dehydrated potato-dextrose agar of different H-ion concentrations: A, At pH 4; B, at pH 5; C, at pH 5.5; D, at pH 6. The original inoculum in the form of a sclerotium is still visible in the center of each colony; compare with Figure 3

similar composition is not entirely clear, but there can be little doubt that the dehydrating process has changed the substance to such an extent as to suggest a marked difference in the amount and kind of nutrients as well as in buffer capacity. The buffer index was undoubtedly different in the two media. The writers' notes show, for example, that with the same initial H-ion concentration it took 0.2 c. c. and N 1 HCl to bring 100 c. c. of the ordinary potato-dextrose agar to a H-ion concentration of pH 5 when 0.35 c. c. of N 1 HCl was required to bring 100 c. c. of the dehydrated medium to the same end point. It would thus appear that the dehydrated material was

much more highly buffered, and this may in part explain the difference in growth. It may be concluded then from these tests that the OH-ion content by itself is not as highly specific in inhibiting growth as was indicated in the former experiments and that the buffer capacity, involving in this case the freedom of activity of the OH ions, has considerable influence on growth response.

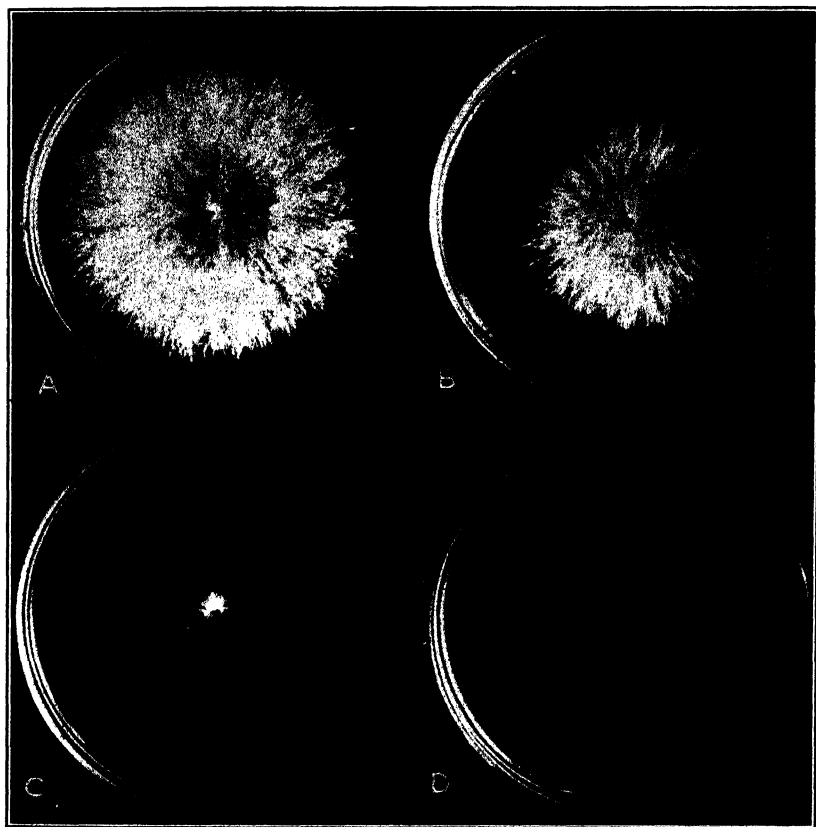


FIGURE 3.—Ninety-six-hour growth of *Sclerotium rolfii* on dehydrated potato-dextrose agar at different H-ion concentrations: A, At pH 6.5; B, at pH 6.9; C, at pH 7.5; D, at pH 8.5. Note the marked decrease in growth around the neutral point and beyond that into the alkaline range, with complete inhibition at pH 8.5; compare with Figure 2

METABOLIC INTERCHANGE BETWEEN *SCLEROTIUM ROLFSII* AND *FUSARIUM VASINFECTUM*

The possible influence of *Fusarium vasinfectum* in stimulating the growth of *Sclerotium rolfii* on alkaline media has already been mentioned. It seemed worth while to investigate this problem more thoroughly. Three series of plates were run simultaneously, dehydrated potato-dextrose agar being used. The first series, which has just been described, was designed to determine the growth relationship of *S. rolfii* to H-ion concentration when no other microorganism was present; the second was to determine the same thing for another soil inhabitant, *Fusarium vasinfectum*; and the third consisted in

bringing together the two fungi at different levels of H-ion concentration in order to determine any possible interaction between the two. Altogether the three series consisted of 125 plates involving in most instances a triplication in any one test.

The medium was well adapted to promote growth of *Fusarium vasinfectum*, the colonies when grown alone developing well, with of course some differences, in practically the whole range of H-ion concentrations under investigation, from pH 3 to pH 9. But when

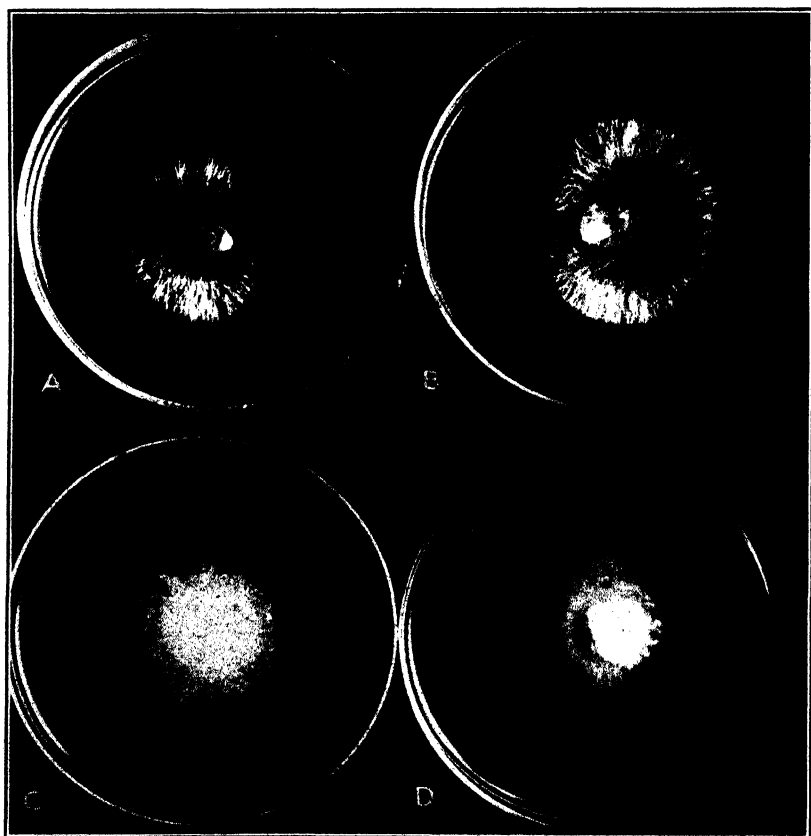


FIGURE 4.—Ninety-six-hour growth of mixed cultures of *Sclerotium rolsfii* (coarse mycelium) and *Fusarium vasinfectum* (fine, compact mycelium) on dehydrated potato-dextrose agar at different H-ion concentrations: A, At pH 4; B, at pH 5.5; C, at pH 6.9; D, at pH 8.2. Note the preponderance of *S. rolsfii* on the acid media and the overwhelming growth of *F. vasinfectum* around the neutral point and on the alkaline side; at pH 6.9 the sclerotial inoculum is so overrun by the *Fusarium* as to be hardly observable

inocula of the two fungi were introduced into the same plate at approximately the same time and placed near each other, markedly different effects were observed. In the acid media below pH 6.9, *S. rolsfii* completely outgrew the *Fusarium*, so that within the space of a 4-day interval the latter, while still noticeable as a small pinkish, compact colony, was almost completely surrounded by the whitish, coarse growth of the *Sclerotium*. (Fig. 4.) As the period of incubation was increased so much of the *Fusarium* on the acid media was

covered by the *Sclerotium* that, had it not been for the marked color production of this strain, it would have been entirely lost sight of. (Fig. 5, C.) Whether *Fusarium* was killed by this overgrowth was not determined.

The reverse phenomenon occurred near the neutral point and in the alkaline ranges. At pH 6.9 and above the sclerotia failed at first to germinate and within four days the fusarial colonies had found their

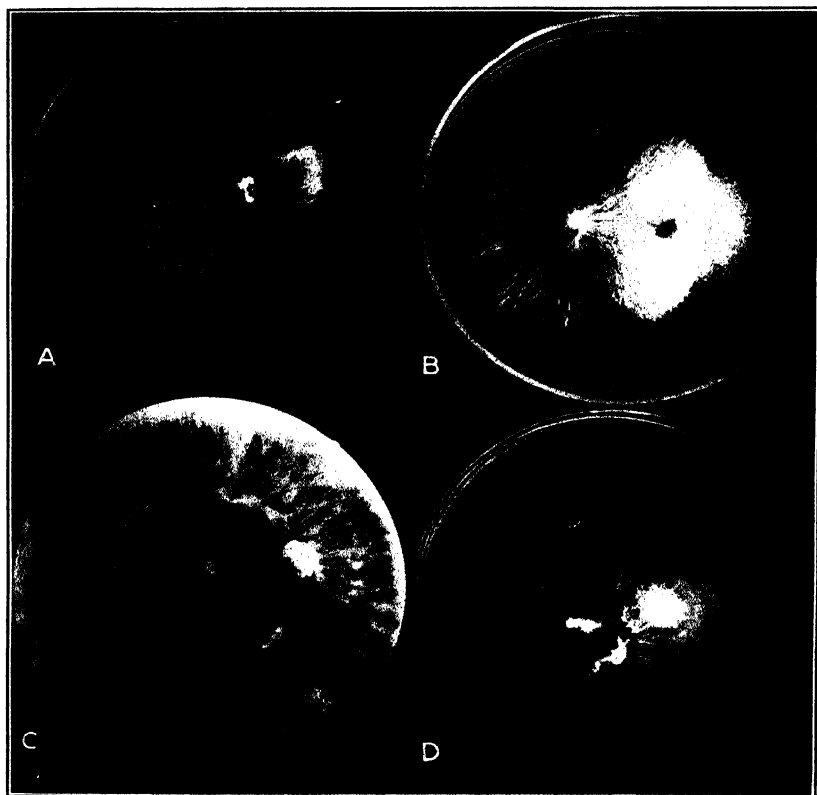


FIGURE 5.—The interaction of soil inhabitants when introduced on a potato-dextrose agar medium which had previously sustained growth of one of the fungi. Following a 4-day growth of *Fusarium vasinfectum*, observable at the right of each plate, there is a 2-day growth of *Sclerotium rolfsii* on A, B, and D, the media having originally H-ion concentrations as follows: A, pH 3; B, pH 5; D, pH 9. C is the same as B of Figure 4, or pH 5.5, photographed 48 hours later and showing the complete overrunning of the *Fusarium* by *Sclerotium*, on acid media when the two are grown simultaneously. Note the overrunning of the *Fusarium* colonies in A and B and the shunning of the medium immediately surrounding the *Fusarium* by *Sclerotium*. Contrast this with D, where, in an alkaline medium, *Sclerotium* is growing immediately around the *Fusarium* colony as well as upon it.

way under the sclerotia or had grown over them so completely as to hide them from sight. (Fig. 4.) Eventually a few of the sclerotia sent out some feeble hyphae, but these were never able to compete successfully with those of the *Fusarium*. It is of course conceivable that if the Petri dishes had been of greater depth and had contained more media, which would not have dried out as rapidly as in the ordinary plates, the sclerotia might in time have overcome the initial retardation in growth, but if one attempts to envision the

possible behavior of the two fungi under natural field conditions, given H-ion concentrations similar to those under discussion, it is difficult to see how under the ordinary weather conditions prevalent in the South the sclerotia would be more favored than in these artificial cultures. On the other hand, with exceptionally moist periods, it is entirely possible that it might be.

Summing up the results obtained in the interaction of the two fungi when inoculated simultaneously, it may be noted that in the acid ranges *Fusarium vasinfectum* is markedly suppressed while in the alkaline ranges *Sclerotium rolfsii* has suffered. Comparing this with the growth responses of the two when grown separately, it is seen that the effect is mostly on the *Fusarium*, while *Sclerotium* has not been greatly influenced.

The next question is what influence will these fungi have on each other when one is introduced some time after the other has had an opportunity to make growth. To determine this point the following experiment was made. Colonies of *Fusarium vasinfectum* were grown for four days in triplicate on dehydrated potato-dextrose agar possessing the reactions pH 7.5, 8.2, 8.5, and 9. At the end of 96 hours, when fair-sized colonies of this fungus had developed, a single sclerotium of *S. rolfsii* was introduced into each plate close to the margin of the *Fusarium* colony. After an incubation of 48 hours the sclerotium in each instance was found to have germinated. In most cases the *Fusarium* colony had continued its growth and had even pushed a flat wedge of hyphae beneath the sclerotium, but this in no way interfered with the germination of the sclerotia and with the subsequent development of the colony. It thus appears that the changes in the medium induced by *Fusarium* permitted the germination and growth of sclerotia in a time interval which is entirely inadequate to permit growth when the sclerotia are used alone. An inspection of Table 2 shows that no growth occurred on media testing pH 8.2, 8.5, and 9 after 72 hours of incubation. A study of other plates of this series which had also sustained growth of *Fusarium* for four days was made to determine any possible change in reaction of the medium, and in every instance it was found that the reaction of the medium adjoining the colony had changed toward the acid side of the H-ion concentration. There may have been other substances which stimulated the sclerotia of *S. rolfsii* into growth, but in the absence of any knowledge of such stimulatory substances and in view of the fact that *Fusaria* instead of producing such materials are known to give rise to staling products that are inhibitory to growth (19), it seems reasonable to conclude that the change in reaction of the medium was primarily responsible for the immediate growth of the sclerotia.

MORPHOLOGICAL RESPONSES OF SCLEROTIUM ROLFSII IN MIXED CULTURES

Up to the present we have been concerned mostly with the rates of growth of *Sclerotium rolfsii* when grown alone or in the presence of *Fusarium vasinfectum*. Among other influences which remain to be detailed are the peculiar types of growth encountered in *S. rolfsii* when this fungus is brought in contact with *Fusarium*. One of these is the apparent chemotropic response of *S. rolfsii* to a *Fusarium* colony in

media of relatively high acidity. *Sclerotium* in such instances seems to shun the substratum immediately surrounding the *Fusarium*, and produces a profuse growth on top of the *Fusarium*. (Fig. 5, B.) In alkaline media this does not occur, the *Sclerotium* growing indiscriminately on the substratum around the *Fusarium* as well as upon the latter fungus. These peculiarities were noted when *Sclerotium* was introduced on plates in which the *Fusarium* had grown for four days; the chemotropic type of growth was observed particularly at pH 3.0 to pH 5.0, while above this no such effects were detected.

In the light of the previously noted effects on the substratum, the following explanation is suggested for this response. On the strongly acid media the change in reaction toward alkalinity in the vicinity of the *Fusarium* colony rendered these places unsuitable for immediate growth of the *Sclerotium*, so that, taking the course of least resistance, the invader grew over the other fungus. It is to be doubted whether the *Fusarium* mycelium served as nutrient material for the *Sclerotium*.

Another type of morphological response noted in *Sclerotium rolfii* when it grew on top of the *Fusarium* colony was the production of a distinct arborescent type of growth in contrast to the diffuse, fluffy growth which it usually makes on artificial media. (Fig. 6, D, and fig. 7.) There appears to develop a definite orientation of the hyphae into main axes and side branches, and these in turn give rise to sclerotia which develop along the sides and tips of the branches. (Fig. 7, B.) When examined microscopically the hyphae in such cases are seen to run parallel to each other and to be more or less compacted into a single unit. The reason for this response is not clear, but it is quite comparable to the form of growth frequently found in this fungus when it is growing in its natural habitat on soil. This may perhaps be attributed in part to the competition offered by other soil inhabitants in which *Sclerotium* is able to hold its own by its ability to produce a compact growth.

The arborescent growth may be compared to the secondary mycelium of higher Basidiomycetes. According to Gäumann and Dodge (4, p. 403)—

the differentiation into main axes and branches, the second characteristic of secondary mycelium, is easily noted in artificial cultures. The main axes are well developed; in contrast to hyphae of the uninucleate [primary] mycelium they run almost parallel to each other and lend themselves easily to the formation of rhizomorphs.

Another possible explanation of the production of this arborescent growth is that the fungus is about to produce fruiting bodies and that this growth is analogous to the tertiary mycelium of higher Basidiomycetes. In the latter Gäumann and Dodge (4, p. 404-405) state—

the secondary mycelium does not proceed as such to the formation of basidia but its hyphae intertwine with extensive change of form and often with loss of individuality to form fructifications, tissues, and organs which in their structure and functions are specialized like those of the Cormophyta. All these tissuelike hyphae systems (plectenchyma, etc.) which have grown from the original, uniform, secondary mycelium are called tertiary mycelia, and develop either as rhizomorphs and sclerotia or as fructifications.

Irrespective of the designation of this type of growth as secondary or tertiary mycelium, its occurrence lends appreciable support to the idea that this fungus may be classed with the higher Basidiomycetes. Summing up the data which point to such relationships

we find (1) that the main vegetative body is made up of hyphae possessing clamp connections; (2) that the cells of this body are binucleate, as shown by Higgins (6); (3) that this fungus readily utilizes cellulose for nutritive purposes, a feature possessed by many Basidiomycetes; and (4) that it produces under certain conditions an arborescent growth which is differentiated into main axes and side branches.

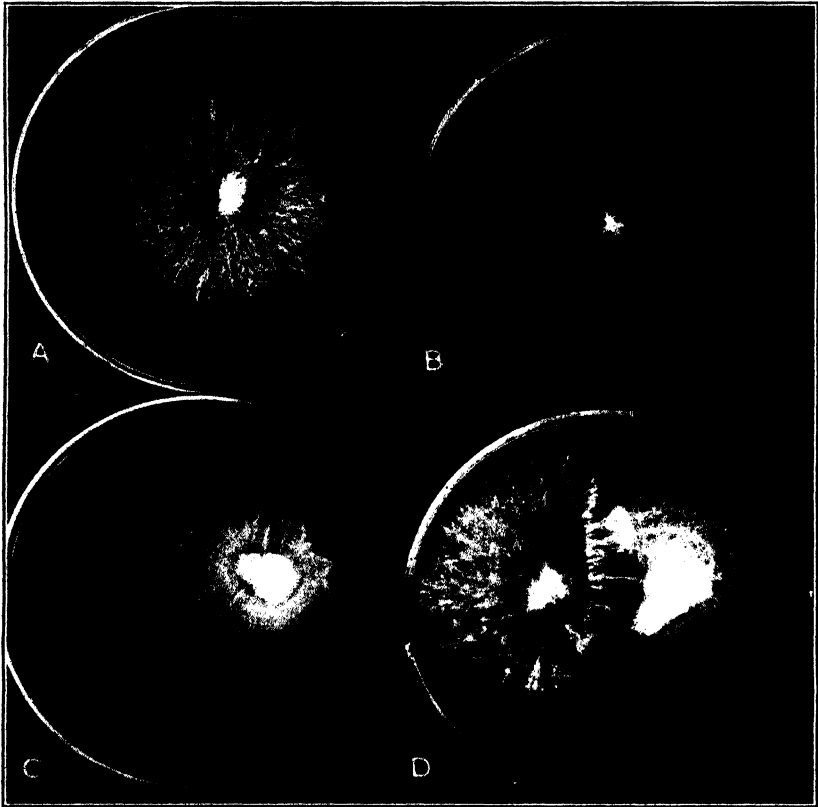


FIGURE 6.—Influence of quantity and type of inoculum in counteracting unfavorable alkaline media; all plates originally tested pH 8.5. A, Forty-eight-hour growth of *Sclerotium rolfsii* when inoculated in form of a relatively large piece of actively growing mycelium; B, 6-day growth of the same fungus when inoculated in form of a sclerotium; C, 4-day growth of *Fusarium vasinfectum* followed by inoculum of *S. rolfsii* in the form of a sclerotium, photographed two days after the introduction of the latter; D, same as C except that the inoculum of *S. rolfsii* was in the form of a large-sized piece of actively growing mycelium. Note the distinct enhancement in growth wherever the inoculum was large and consisted of actively growing mycelium.

Using the technic of Stevens (24), by which he was able to bring about fruiting in various fungi, including the perfect stages of Ascomycetes, through the action of ultra-violet rays, the writers attempted to induce spore formation in *Sclerotium rolfsii*. Five Petri-dish cultures of this fungus were subjected to ultra-violet rays for approximate time intervals of one-fourth, one-half, 1, 10, and 20 seconds.⁶ The plates were held at a distance of about 21 centimeters

⁶ Through the kindness of D. M. Moore, of the botany department, University of Arkansas, there was placed at the writers' disposal a quartz mercury arc lamp.

from the light, with one-half of each covered by a black shutter from a photographic plate holder. The colonies were 12 days old at the time of treatment and had completely covered the media. No fruiting bodies were observed on these plates up to 34 days after the ultra-violet application, the media in most instances having almost completely dried out by that time. However, in one plate where drying out had not occurred (the one irradiated for 20 seconds) there was a noticeable fluffy or fuzzy growth covering the sclerotia on the exposed portion of the plate. When examined under the microscope this fluffy material was seen to consist of loosely interwoven and partly free hyphae. But in no case was there any suggestion of basidia formation or of any other spore-forming organs. A careful study of these sclerotia showed that they had failed to mature properly, putting down around the periphery no hardened cells of a brownish color as occurs in normal cases. There appeared also to be a lack of differentiation into a plectenchyma and a central core.

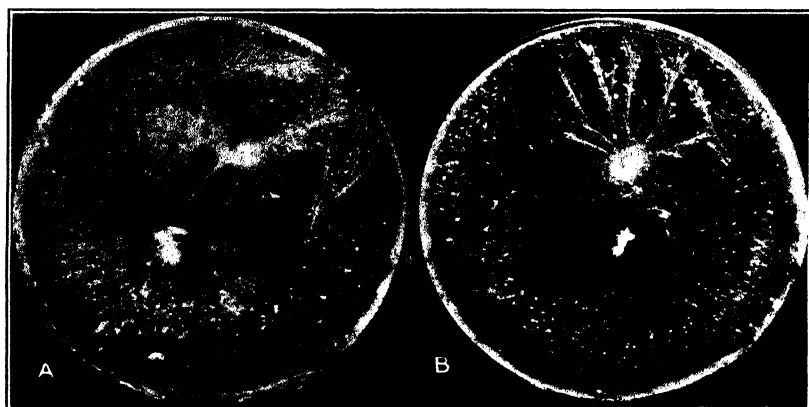


FIGURE 7.—Arborescent type of growth of *Sclerotium rolfsii* when it is growing upon a *Fusarium* colony. A is the same as D of Figure 6 but photographed six days later. Note how *Sclerotium* has almost completely overgrown and obliterated from view the underlying *Fusarium* colonies. The arborescent growth is only visible in those parts of each plate where the *Fusarium* colony existed

Altogether, the apparent effect produced by the ultra-violet rays on these sclerotia was to keep them in a juvenile condition. These results are offered as suggestive, however, and not as final. There is, of course, the possibility that these bodies, which in gross aspects resemble sclerotia, are either morphological entities capable of giving rise to a spore-forming stage when conditions are favorable or that they represent abnormal growths brought about by the action of the ultra-violet rays.

Quite recently Nakata (12), working in Japan, reported that he had found the perfect stage of *Sclerotium rolfsii* to be *Corticium centrifugum* (*Hypochnus centrifugus*). He undertook a series of morphological and cultural studies of 33 strains of *S. rolfsii* and succeeded in obtaining the perfect stage in two strains from Japan and in one from America. A summary of his results, presented in English, indicates that the spore-forming stage is very similar to *Corticium solani* (*C. vagum*) and that the main differences are to be found in the mycelium and in sclerotial development.

THE INFLUENCE OF RELATIVELY LARGE QUANTITIES OF INOCULUM OF *SCLEROTIUM ROLFSII* IN COUNTERACTING UNFAVORABLE ALKALINITIES

The work recorded previously in attempting to determine the growth reactions of *Sclerotium rolfii* when grown alone or jointly with another fungus was, with the exception already noted, carried out entirely with inoculum consisting of sclerotia. During the course of these investigations there appeared the possibility of inducing growth in this fungus on media inhibitory to the germination of sclerotia but capable of promoting growth of active mycelium. In order to test this out the writers inoculated a series of plates containing approximately equal quantities of dehydrated potato-dextrose agar which had been rendered alkaline by the addition of NaOH. The range in H-ion concentration from pH 8.2 to pH 9, which had previously been found to prevent sclerotial germination for 72 hours (Table 2), was utilized, and the inoculum consisted of relatively good-sized pieces of actively growing mycelium. These transplants were cut out in the form of about 8-mm. squares from tubes containing the same medium, the squares of inoculum consisting of underlying substratum as well as mycelium. Within 24 hours a vigorously growing colony had developed in the entire reaction range, and within 48 hours the growths in most cases covered more than half of the available growing area. (Fig. 6, A.) With the exception of the extreme range of pH 9, no marked difference in growth rates could be detected, and even at pH 9 the growth at the end of 48 hours was fully as good as that obtained in most of the acid ranges in the same time interval when a single sclerotium was used as inoculum. It may therefore be concluded that the type and quantity of inoculum are very important factors in initiating growth and, given the proper substrata and a suitable inoculum, growth may be expected to occur irrespective of the reaction.

SUMMARY

There exists very little information at present on the influence of other microorganisms present in the soil on the growth and activities of soil-inhabiting plant pathogens.

The very severe epidemic of *Sclerotium cantaloupe* rot in Arkansas in 1928 suggested the desirability of utilizing *Sclerotium rolfii* as one of the fungi to be studied. The outstanding features of this epidemic are discussed and evidence is presented to show that while cantaloupe fruit was the only plant part found infected in the field, the strains isolated from these fruits could readily parasitize other plants and other plant parts.

It is shown that *Sclerotium rolfii* can easily utilize cellulose for nutrient purposes.

Studies on the growth of *Sclerotium rolfii* on potato-dextrose agar at various H-ion concentrations reveal the fact that the fungus on this medium is markedly tolerant of acid conditions and is quite sensitive to alkaline reactions. There is also a noticeable gradient in sclerotial development on acid media. Beginning with pH 8 and extending upward, sclerotia failed to germinate on this medium.

Fusarium vasinfectum, the other soil inhabitant here studied, grew well on this medium throughout the range of H-ion concentration under investigation, that is, from pH 3 to pH 9.

Since *Fusarium vasinfectum* is known to change the reaction of a substratum upon which it is growing, turning a strongly acid medium toward alkalinity and an alkaline medium toward acidity, this fungus was grown on potato-dextrose agar having those alkaline reactions of pH 8 to pH 9 which had inhibited growth of sclerotia. When reinoculated with *S. rolfsii* these Petri-dish cultures showed abundant growth of the latter fungus.

In order to determine the responses of these microorganisms to a standardized medium of similar composition and to check the results of the previous experiments, use was made of a commercial dehydrated potato-dextrose agar. *Sclerotium rolfsii*, in quantities of inoculum similar to those used in the previous tests, made a greater growth on this medium than on ordinary potato-dextrose agar throughout the range of H-ion concentration investigated, from pH 3 to pH 9. For the first three days the sclerotia failed to germinate on the media testing pH 8.2 to pH 9, but eventually they made growth on these.

The dehydrated product was found to have a much higher buffer capacity than the homemade potato-dextrose agar, and it is concluded that the hydroxyl-ion content by itself is not as highly specific in inhibiting growth of *Sclerotium rolfsii* as was indicated in the former experiments.

Dehydrated potato-dextrose agar was also found to be well adapted to promote growth of the cotton-wilt fungus, *Fusarium vasinfectum*.

When the two fungi were grown simultaneously on the same plate containing the dehydrated medium, different effects were obtained. In the acid media below pH 6.9 *Sclerotium rolfsii* completely outgrew and in time entirely covered the *Fusarium*, while around the neutral point and in the alkaline ranges the reverse phenomenon occurred.

With the introduction of *Sclerotium rolfsii* into plates that had previously sustained growth of *Fusarium vasinfectum*, still other results were obtained. In such cases dehydrated media, originally rendered alkaline, permitted the germination of sclerotia in time intervals that are unsuitable for germination when *Sclerotium rolfsii* is grown by itself.

Negative chemotropic responses were noted in *Sclerotium rolfsii* on media originally possessing acid reactions which had, prior to the introduction of this fungus, been used for growing *Fusarium vasinfectum*. This response resulted in the overgrowing of *Fusarium* when the surrounding medium remained uninhabited.

The growing of *Sclerotium rolfsii* on top of a *Fusarium* colony resulted in an arborescent growth of the former consisting of the production of main axes and side branches. These in turn were found to be made up mostly of parallel running hyphae.

Arborescent types of growth are noted to be common when *Sclerotium rolfsii* grows naturally on soil. Attention is directed to the fact that this type of growth may offer a valuable clue to the relatives of this fungus, and several points are enumerated which indicate its possible relationship to the higher Basidiomycetes.

When attempts were made to induce spore formation in *Sclerotium rolfsii* by means of ultra-violet rays, negative results were obtained.

It was found that the type and quantity of inoculum of *Sclerotium rolfsii* are very important factors in overcoming reactions that are unfavorable to the germination and growth of sclerotia.

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THE RATE OF SPREAD OF POTATO VIRUS DISEASES IN WESTERN NEBRASKA¹

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INTRODUCTION

The rate and distance of the spread of potato virus diseases has been the subject of considerable speculation and some experimentation because of its great importance in the production of disease-free seed potatoes. Various investigations have shown that both rate and distance of spread will vary in different localities and in successive seasons in the same locality. It is therefore impossible to forecast the amount of spread in one locality from the results of investigations conducted under different environmental conditions. The only experiments dealing with the subject which have been conducted under western dry-land or irrigated culture are those reported by Werner³ in a study of the spindle-tuber disease.

Spindle tuber has for some time been recognized as the most prevalent virus disease in western Nebraska; the various mosaics are not so common; and leaf roll seldom occurs. This order of relative importance is the reverse of that observed in the eastern part of the United States.⁴ It was therefore thought probable that the factors involved in the spread of these diseases in this western area were probably very different from those occurring in the East.

The present investigation was outlined to determine if possible the reasons for the above facts and to gain information which might be of value in the control of these diseases. Repeated observations have shown that these diseases often spread more rapidly under irrigated than under dry-land conditions, and therefore the experiment was designed to include both types of culture. Unfortunately the dry-land plot had to be discontinued after the second year, but the results obtained from studying the relative spread of four potato virus diseases—leaf roll, mild mosaic, rugose mosaic, and spindle tuber—under irrigation are presented for the four years 1924 to 1927, inclusive.

METHODS OF INVESTIGATION

Two different methods of experimentation have been employed in previous investigations of this type: (1) The sampling of commercial fields at different distances from the source of infection, as used by Folsom⁵ et al., and (2) the replanting hill by hill, in the same relative position, for several successive generations, of plants which have

¹ Received for publication Feb. 20, 1929; issued July, 1929.

² The author is indebted to R. W. Samson for the survey and identification of insects in 1925 and 1926, and to Karl Koch for similar assistance in 1927. Published with the approval of the director as paper No. 72, Journal Series, Nebraska Agricultural Experiment Station.

³ WERNER, H. O. THE SPINDLE-TUBER DISEASE AS A FACTOR IN SEED POTATO PRODUCTION. Nebr. Agr. Expt. Sta. Research Bul. 32, 128 p., illus. 1926.

⁴ FOLSOM, D., SCHULTZ, E. S., and BONDE, R. POTATO DEGENERATION DISEASES: NATURAL SPREAD AND EFFECT UPON YIELD. Me. Agr. Expt. Sta. Bul. 331, 112 p. 1926.

⁵ FOLSOM, D., SCHULTZ, E. S., and BONDE, R. Op. cit.

been exposed to infection, as employed by Murphy⁶ in the study of leaf roll. The chief advantage of the first method lies in the fact that the diseases are being studied under practical field conditions. This advantage is sacrificed in the second method for the greater accuracy made possible by the more detailed study under conditions having fewer variable and uncontrolled factors. The latter method was used in this investigation.

The investigation was carried out at the Scotts Bluff substation, Mitchell, Nebr. The irrigated plot was isolated in a cornfield each year and was at least 100 to 300 feet distant from the nearest potato field. It was impossible to arrange the plot so that the direction of spread could be studied for successive years. The dry-land plot was located on the same farm but was on a lighter soil to which no irrigation water was applied. It was bordered by fields of corn, wheat, and alfalfa.

The plots used were small in size and consisted of 12 rows spaced 3 feet apart, with 27 hills to the row spaced 18 inches apart. Healthy Bliss Triumph tubers which had been indexed in the greenhouse were used as the source of seed. The term "greenhouse indexing" as used in this paper refers to the removal of one seed piece from a potato. This seed piece was planted in the greenhouse during the winter under conditions favorable for the development of the symptoms of the disease being studied. The remainder of the tuber was planted in the field the following spring. Seed pieces from each tuber were planted in both the dry land and irrigated plots.

Seed tubers obtained from plants affected with the virus diseases to be studied were planted in each plot as follows: Four leaf-roll plants were grown in row 4 as hills 4, 5, 6, and 7 and four mild-mosaic plants as hills 21, 22, 23, and 24. In the same way four rugose-mosaic plants were grown in row 9 as hills 4, 5, 6, and 7 and four spindle-tuber plants as hills 21, 22, 23, and 24. Thus the sources of infection for these four virus diseases, which will hereafter be referred to as the original disease units, were located, one in each corner of the plot. These original disease units are represented in Figures 1 and 2 by the dotted circles.

At the time this experiment was started the distinguishing symptoms of spindle-tuber and the very similar disease unmottled curly dwarf had not been clearly defined in the Bliss Triumph variety. As a result the original spindle-tuber units may have included some unmottled curly dwarf which had simply been diagnosed as severe spindle tuber. In this paper the term "spindle tuber" is therefore used to include both diseases.

One of the usual difficulties in an experiment of this type is the partial or incomplete infection of a hill. To eliminate this trouble as far as possible, each hill was cut back to a single stalk every year. Notes on the symptoms appearing in the field were made at one to two week intervals. Each hill was harvested separately, and a record was made of all symptoms of spindle tuber appearing in the tubers. One tuber from each hill was then selected for indexing in the greenhouse. In some instances a hill had to be classified as questionable spindle tuber, in which case the tuber exhibiting in the

⁶ MURPHY, P. A. INVESTIGATION OF POTATO DISEASES. Canada Expt. Farms, Div. Bot. Bul. (2) 44 86 p., illus., 1921.

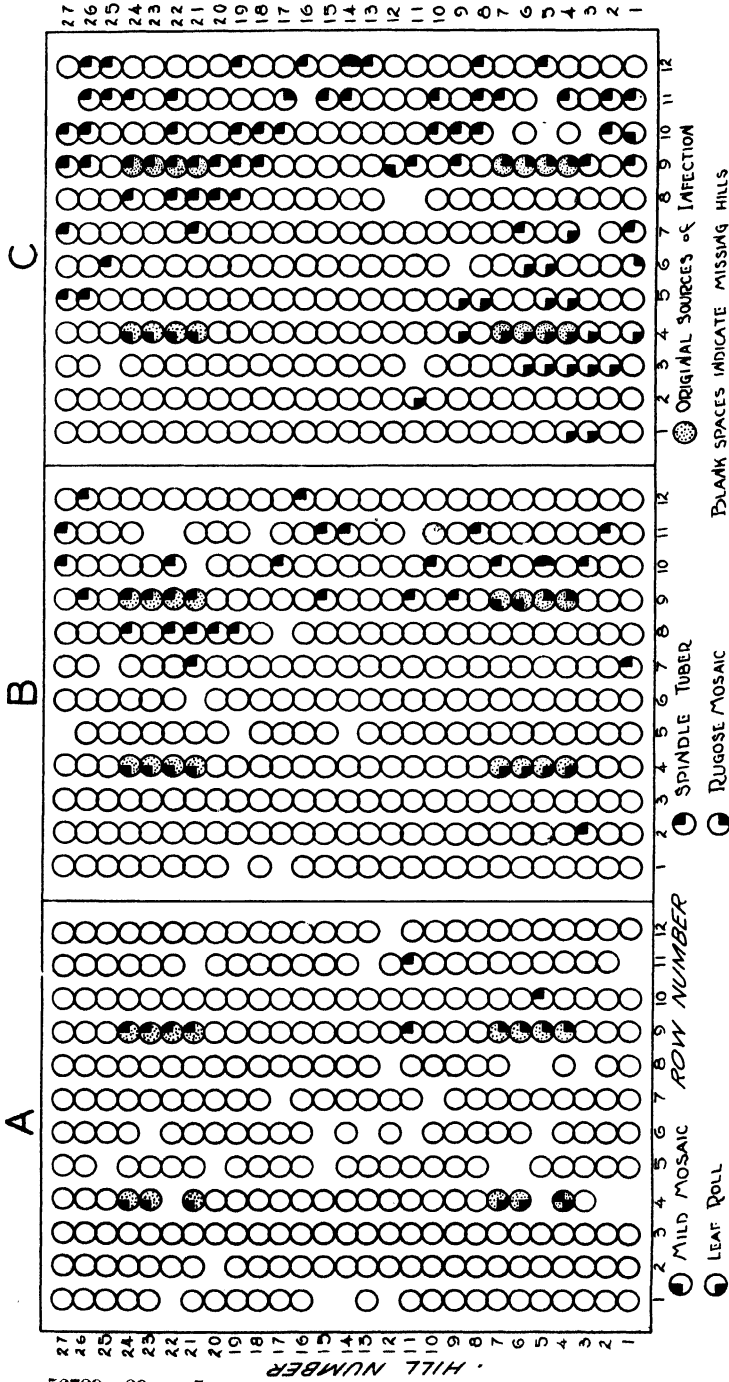


FIGURE 1.—Diagrammatic representation of the irrigated transmission plot for the years 1924 (A), 1925 (B), and 1926 (C). Each circle represents a hill planted in 1924 with healthy seed, except as noted by symbols. Each hill was cut back to a single stalk and one seed piece from each was planted in the same position the following year. The spread of the diseases studied can be followed by attention to the symbols. The plants marked as infected are those having symptoms in the field, but this does not include late-season infection as revealed by indexing (cf. Table 1). Such plants are listed in this figure for the following year when the disease appeared in the field.

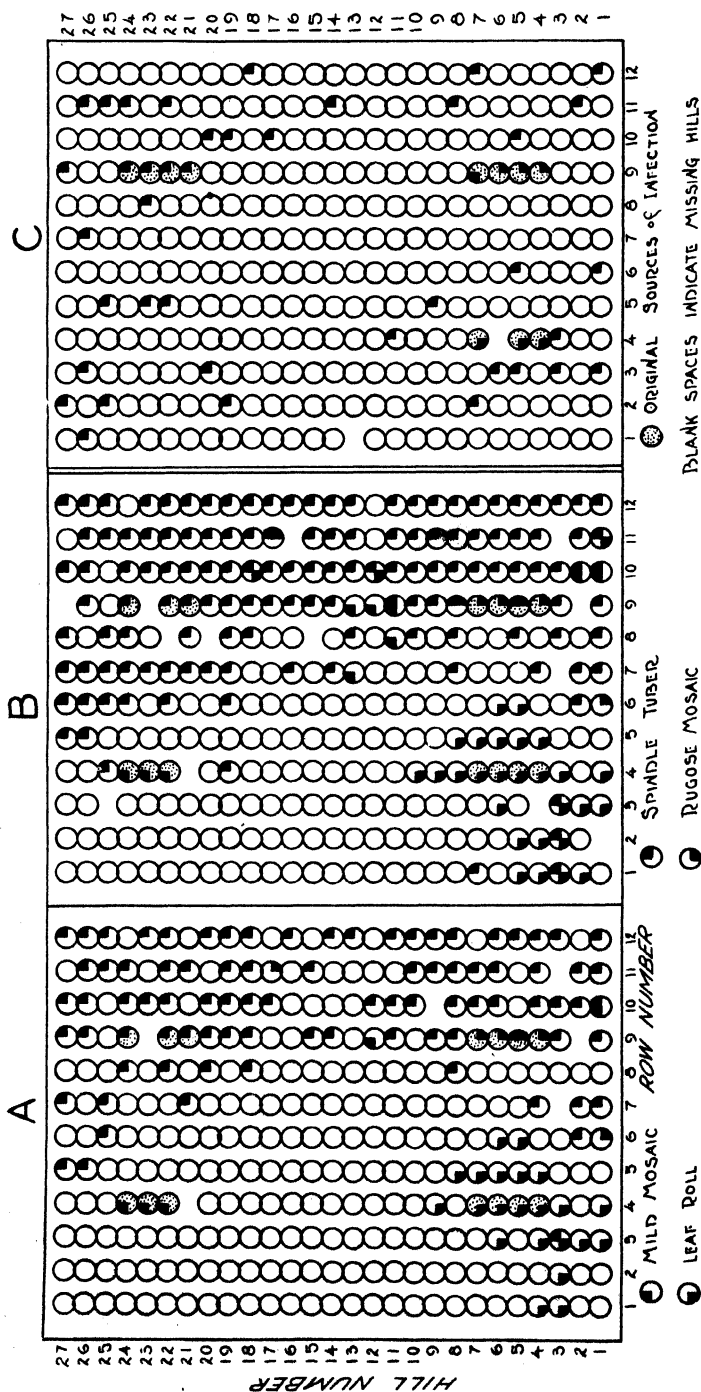


FIGURE 2.—Diagrammatic representation of the irrigated plot for the year 1927 (A); results of indexing the tubers in the greenhouse (B); the dry-land plot for 1925 (C) in which the tubers as original sources of infection in 1924 were planted in a manner similar to that used for the irrigated transmission plot. See Figure 1 for further explanation

greatest degree symptoms resembling those of spindle tuber was selected for indexing. Readings were made in the greenhouse for mosaic, leaf roll, and spindle tuber, and the plants were kept until the tubers had developed sufficiently for the detection of spindle-tuber symptoms. This usually required from two to two and a half months. This greenhouse indexing, which was carried out each year except 1926, was found to be a very great aid in the detection of leaf roll and mosaic, particularly the latter, which, because of the masking of symptoms under high-temperature conditions, is often very difficult to detect in the field in western Nebraska. The indexing in the greenhouse also served to determine the amount of transmission that occurred late in the season of the previous year but did not produce current-season symptoms.

The same tuber indexed in the greenhouse was used for planting in the field the following year. Each seed piece was planted in the plot in the same relative position. In case of missing hills due either to disease or to other causes the hill was planted with healthy seed the following year.

In 1925 and 1927 weekly notes were made of the prevalence of all insect species appearing in the plot. In 1924 and 1926 general observations were made of the insect population at various times throughout the season. No application of either spray or dust was made at any time during the four years.

RESULTS

The progressive spread of the diseases in the plots is shown graphically in Figures 1 and 2. The plants listed as infected are those which exhibited symptoms of the specified disease, either in the greenhouse the previous winter or in the field, and with only one exception the progeny of these hills showed similar symptoms. These plants may therefore have been the result of late infection the previous season or early current-season infection.

In the data presented in Table 1 and in the following discussion, the attempt has been made to determine the time of infection on the basis of the results secured by indexing in the greenhouse. Because of the above facts the figures presented in Table 1 and in the discussion do not check exactly with the data presented in Figures 1 and 2. The inclusion in the diagrams of data on the time of infection would have resulted in a complicated set of symbols and has therefore been omitted.

During 1924 and 1925 no precautions were taken against the spread of these diseases by the cutting knife. In 1924, however, the healthy seed was cut before the infected seed so that no disease could have been transmitted to healthy seed in this way. In 1925 there was no evidence of knife transmission having occurred as all seeds were cut in the same sequence as planted and no infection occurred in the hills immediately following the infected hills of the previous year. This lack of transmission by the cutting knife was probably due to the fact that the seed was cut a considerable time in advance of planting.

By examining Figures 1 and 2 it will be found that many plants listed as infected one year are marked as healthy the following year. In every instance but one this is due to missing hills or to the replant-

ing of infected hills with healthy seed because of an absence of seed tubers. These missing hills are noted in Table 1.

TABLE 1.—Comparative yearly increase of leaf roll, mild mosaics, rugose mosaic, and spindle tuber in a plot of healthy potatoes grown under irrigation and originally planted with one 4-hill unit of each disease in 1924

LEAF ROLL						
Year	Number of hills ^a	Total of infected hills ^b		New infections		Healthy replants or missing hills of infected seed
		Number	Per cent	Number	Per cent of healthy hills	
1924	295	3	1.0	0	0.0	1
1925	311	14	4.5	10	3.3	0
1926	312	26	8.3	12	4.3	0
1927	318	31	9.7	9	3.0	4

MILD MOSAIC						
Year	Number of hills ^a	Total of infected hills ^b		New infections		Healthy replants or missing hills of infected seed
		Number	Per cent	Number	Per cent of healthy hills	
1924	295	3	1.0	0	.0	1
1925	311	8	2.6	2	.7	0
1926	312	6	1.9	0	.0	0
1927	318	10	3.2	5	1.6	1

RUGOSE MOSAIC						
Year	Number of hills ^a	Total of infected hills ^b		New infections		Healthy replants or missing hills of infected seed
		Number	Per cent	Number	Per cent of healthy hills	
1924	295	4	1.3	0	.0	0
1925	311	6	1.9	4	1.3	0
1926	312	7	2.2	0	.0	1
1927	318	7	2.2	1	.3	0

SPINDLE TUBER						
Year	Number of hills ^a	Total of infected hills ^b		New infections		Healthy replants or missing hills of infected seed
		Number	Per cent	Number	Per cent of healthy hills	
1924	295	13	4.4	9	3.1	0
1925	311	35	11.2	24	8.0	2
1926	312	55	14.4	21	9.1	6
1927	318	135	42.4	87	37.6	6

^a Three hundred and twenty-four seed pieces were planted each year.

^b The total number of infected hills includes those showing symptoms when indexed.

^c Two additional mild-mosaic plants were accidentally introduced in place of two rugose-mosaic plants.

^d No indexing was done in 1926. Therefore part of those listed as 1927 infections may have occurred in 1926. This error does not affect the mild and rugose mosaic readings, as these infections occurred late in 1927.

The following discussion of the spread of these diseases refers entirely to the irrigated plot unless otherwise stated.

LEAF ROLL

The seed in one hill of the original leaf-roll unit planted in 1924 failed to sprout, while a second became infected with spindle tuber, possibly by the cutting knife. These were replaced in 1925 by other seed infected with leaf roll. No spread of leaf roll occurred in 1924. In 1925 no additional leaf-roll plants appeared in the field, but evidently some spread occurred, as 10 new leaf-roll plants were found when the progeny were indexed in the greenhouse. The infection probably occurred late in the summer of 1925. In 1926, 22 leaf-roll plants were found. Of these, 4 were in the original leaf-roll unit while 10 were due to previous season infection as noted above. The remaining 8 plants all showed slight symptoms of leaf roll early in July and were easily identified as leaf-roll plants by the 10th of August.

The disease was evidently caused by early season transmission. In 1927 only 4 new leaf-roll plants were found and these appeared as distinctly diseased early in July. The symptoms were as severe and appeared about as early as in the original leaf-roll unit, thus indicating that infection had probably occurred late in the season of 1926, and they are therefore listed in Table 1 as 1926 infections. The greenhouse index of 1927, however, revealed the fact that 9 new infections had occurred during the summer.

It may therefore be concluded that the greatest spread of leaf roll occurred late in the season of 1925. With three times as many sources of infection present in 1926, only 12 new infections occurred. Eight of these were evidently transmitted early and 4 late in the season. Only a slight amount of transmission occurred in 1927, as evidenced by 9 new infections when there were 22 plants to serve as sources of infection.

It is clearly evident from Figures 1 and 2 that the disease did not spread any great distance. Even after four years all the leaf-roll plants were restricted to that corner of the plot containing the original leaf-roll unit and no new infections appeared at any time more than three or four plants removed from leaf-roll plants. There was no apparent difference in spread from row to row and hill to hill within the row.

MILD MOSAIC

Only three of the four seed pieces in the original mild-mosaic unit sprouted in 1924. No transmission from these plants occurred in 1924 and there were no additional mild-mosaic plants found in the field in 1925. Two of the hills planted in 1925 in the original rugose-mosaic unit in row 9 were found to have been mild rather than rugose mosaic so that in 1925 there were six mild-mosaic plants in the plot. Two transmissions occurred, apparently from these two additional sources of infection, the symptoms appearing in the greenhouse and in the field in 1926. (Fig. 1, C, row 9, hill 12, and row 10, hill 1.) No spread of mild mosaic occurred in 1926 and no additional mild mosaic was observed in the field in 1927. Some transmission, however, probably occurred late in the season of 1927 as the indexed plants in the greenhouse revealed the presence of five new infections. Judging from the location of these hills in the plot (fig. 2, B, rows 7, 8, 9, and 10) none of these infections were transmitted from the original mild-mosaic unit but rather from the two new mild-mosaic plants of 1926, which in turn could be traced to the two mild-mosaic plants accidentally introduced in the original rugose-mosaic unit in 1925.

It is evident that in 1924 and 1926 no transmission of mild mosaic occurred and only a slight amount of spread took place in 1925 and 1927.

RUGOSE MOSAIC

Except in 1925, when two mild-mosaic plants were accidentally inserted in place of rugose-mosaic ones, all plants in the original rugose-mosaic unit exhibited the typical symptoms every year. No transmission occurred in 1924 and only one additional rugose-mosaic plant was found in 1925, the symptoms appearing early in September. Three other infections, however, must have occurred late in 1925, as the symptoms appeared on the indexed plants in the greenhouse and in the field in 1926. No new infections were noted in 1926 and

only one in 1927, which probably occurred late in the season as the disease appeared for the first time in the indexed plant. The only instance of partial or incomplete infection of a tuber occurred with hill 14 in row 12 (fig. 1, C), which showed typical symptoms of rugose mosaic in the field in 1926 but failed to show any symptoms of the disease in 1927.

It is clearly evident that only a very slight amount of transmission, five infections, occurred in two of the four years. Two of these were to hills adjoining the original rugose-mosaic unit and the other three were somewhat scattered but none was more than a distance of 12 hills from a rugose-mosaic plant.

SPINDLE TUBER

Although the amount of transmission obtained with the mosaic diseases was slight, spindle tuber spread much more extensively. (Figs. 1 and 2.) The first evidence of spindle-tuber transmission was found when the tubers produced in 1924 were examined. Four plants produced tubers with distinct spindle-tuber symptoms and in addition 5 new infections were found when the tubers were indexed. In 1925 there were 19 new spindle-tuber plants as evidenced by either foliage or tuber symptoms, and 5 additional infections were discovered by indexing. In 1926 there were 26 infections, resulting in the appearance of current-season symptoms on either foliage or tubers. As no index was made in 1926, it is impossible to determine how many of the 38 new spindle-tuber plants that appeared in the field in 1927 were due to transmission late in the season of 1926. The index of the 1927 crop disclosed the fact that in addition to these 38 new spindle-tuber plants there were 49 late-season infections that did not cause symptoms in the tubers but did produce the typical disease when these tubers were indexed.

Even with the large amounts of spindle tuber present after the first year only 42.4 per cent of the plants were infected after four years. Furthermore, the disease did not spread for any great distance after the first year. In 1924, in addition to the original spindle-tuber unit, there were nine scattered infections, four of which are indicated in Figure 1, A. The five additional infections, which were found by indexing and which are listed in Figure 1, B, occurred in hills 1 and 21 in row 7, hill 22 in row 8, hill 9 in row 9, and hill 27 in row 11. Most of the spread in the following years was centered around these plants and the original spindle-tuber unit, with the exception of hill 3 in row 2, which became infected in 1925, and hills 26 and 27 in row 5, infected in 1926. An examination of Figure 2, A and B, might lead one to assume that the spread of this disease occurred chiefly in one direction. As previously noted, however, the plot was not laid out in the same direction each year. It is more probable that the large amount of spread in rows 9 to 12 was due to the distribution of the infections which occurred in 1924 and 1925. (Fig. 1, A, B.) The remarkable thing is that after four years there were only six spindle-tuber plants more than three rows removed from the row containing the original spindle-tuber unit, a fact which showed that the disease did not spread for any great distance. It was also notable that in these four heavily infected rows there still remained a half dozen healthy plants at the end of the four years. These are being tested to determine whether they possess any inherent resistance to the disease.

COMBINATIONS OF DISEASES

It would be expected that in a plot of the type used in these experiments there would be a considerable number of plants infected with more than one disease. It was fairly easy to distinguish disease combinations, with the possible exception of mild mosaic combined with rugose mosaic, and as these two diseases did not spread very extensively it is doubtful that this combination occurred. There were 18 plants infected with spindle tuber in addition to 1 of the other virus diseases, 4 of these were combinations with mild mosaic, 6 were rugose mosaic, and 8 were leaf roll.

RELATION OF INSECTS TO SPREAD

IRRIGATED PLOT

The greatest amount of infection with all four diseases occurred in 1925 and the next largest amount in 1927. Only leaf roll and spindle tuber were transmitted in 1926 and only spindle tuber in 1924.

By checking over the insect survey made on five different dates between July 2 and August 26 in this plot in 1925, it was found that a small number of aphids were present on July 2 and a much larger number on August 26. On the latter date 183 plants were carefully examined and from 1 to 7 aphids were found on each of 38 per cent of the plants. While this could hardly be called a heavy infestation of aphids, it was the largest number found at any time during the four years and probably accounts for the greater amount of transmission that occurred in that year. The other insects noted in 1925 were Colorado potato beetles, gray blister beetles, leaf hoppers, tarnished plant bugs, and grasshoppers. The tarnished plant bugs were the most abundant.

In 1927 the only aphids observed were found very late in the season just before digging. This may explain the fact that the only transmission of mosaic and leaf roll occurred late in the season and was not evident until the tubers were indexed in the greenhouse. A considerable number of flea beetles were present early in July and August, but no other insects were noted except Colorado potato beetles, a few leaf hoppers, and some grasshoppers.

In 1924 and 1926 the records of insect infestations were not made as accurately as in the other years, but a careful lookout was kept for aphids both in this plot and in the other experimental plots on the farm. None, however, were noted.

From these observations it would appear that the few instances of leaf-roll transmission in 1926 and the extensive spindle-tuber transmission in all four years must be explained on some other basis than that of aphid transmission. Grasshoppers,⁷ which have been shown to be capable of transmitting spindle tuber, were possibly the chief agents in the spread of this disease, although the other insects present can not be eliminated until further tests of their ability to transmit the virus have been made.

DRY-LAND PLOT

The type of spread of spindle tuber occurring in the dry-land plot was in sharp contrast to that presented above for the irrigated plot.

⁷ GOSS, R. W. TRANSMISSION OF POTATO SPINDLE TUBER BY GRASSHOPPERS (LOCUSTIDAE). *Phytopathology* 18: 445-448. 1928.

The dry-land plot was only used for two years and the detailed data are only presented for one year, 1925. (Fig. 2, C.) This plot was planted in the same manner as the irrigated plot, both the infected and healthy seed being from the other halves of the seed tubers used for the irrigated plot. Because of unfavorable conditions, the stand was very poor in 1924. Only 207 hills were produced from 324 seed pieces planted. Only two plants in addition to the original spindle-tuber unit exhibited the symptoms of this disease and there was no evidence of spread of the other three diseases.

This plot suffered a severe infestation of grasshoppers during 1924 from an adjoining alfalfa plot, the first three rows being almost entirely defoliated. Grasshoppers were also present in 1925. Figure 2, C, shows the amount of transmission which occurred in 1924 as evidenced by symptoms appearing in 1925 in addition to the current-season symptoms that appeared from transmission early in the season of 1925. There were 40 spindle-tuber plants in 1925. Six of these were present in 1924, and 20 of them occurred in plants grown from healthy seed used to replace the missing hills of 1924, so that not more than 14 of these infections could have occurred late in the season of 1924. This total of 16 possible infections in 1924 is much greater than the number that occurred in the irrigated plot, and the distance of spread is greater than in the irrigated plot even after four years. Undoubtedly the grasshoppers were the chief agents in the spread of spindle tuber. In addition to the 20 new infections that appeared in the field in 1925, there were 16 additional instances of transmission as revealed by indexing the tubers. Most of these infections centered around the plants infected in 1924 and were widely scattered over the entire plot.

In 1925 it was determined by greenhouse indexing that there were two instances of leaf-roll transmission and one of rugose mosaic. This transmission was probably due to late-season infection by aphids, which were present in August, though less abundantly than in the irrigated plot. Neither of these diseases was transmitted in 1924. Due to an error the original mild-mosaic unit was not planted in 1925 and one hill of the original rugose-mosaic unit was accidentally planted with mild-mosaic seed. No transmission of mild mosaic occurred in 1924 from the original unit, nor in 1925 from the single plant in row 9.

DISCUSSION

While the experimental plots used in these tests are not strictly comparable to large commercial fields, the results obtained are in harmony with the writer's observations of the spread of these diseases in western Nebraska. The relative amount of spread of these four diseases in any one year and of each disease for the four years portrays quite accurately the conditions existing in the commercial fields of the same area during the same period.

It is clearly evident that mild and rugose mosaic are not readily transmitted under these conditions. The amount of spread was negligible as contrasted with that observed in the eastern part of the United States by other investigators. Leaf roll, while transmitted more extensively, was still limited in extent and the amount of transmission was much less than that reported by investigators in other sections of the country. These three diseases may be chiefly de-

pendent upon aphid transmission, and therefore have not become as serious a menace under western conditions, where aphids are less frequent than in other parts of the country. Murphy,⁸ however, reports transmission of leaf roll by capsid bugs (*Calocoris bipunctatus*), jassids (*Typhlocyba ulmi*), and flea beetles (*Psylliodes affinis*). It is therefore quite probable that insects other than aphids were responsible for some of the leaf-roll transmission, particularly in 1926, when aphids were not present.

Spindle tuber was found to be quite extensively transmitted, although not to as great distances as reported by other investigations nor in as large amounts as indicated by Werner.⁹ Werner's experiments, conducted in 1923, in which he found a very extensive spread of spindle tuber, are not strictly comparable to the tests reported in this paper, for there may have been in that year a much more severe insect infestation than in any of the following four years. It is clearly evident, however, that this disease is a more serious problem in western Nebraska than the other three diseases studied. The increase from 14 to 42 per cent in 1927 gives some indication of the rapidity with which it may increase when conditions are favorable. It must be remembered, however, that part of this increase probably occurred late in the season of 1926, but was not determined because no indexing was done that year. Judging from the results of these four years' study, which included a considerable range of variation in weather conditions, the disease would hardly be expected to much more than double in amount in an average year. It is also evident that this disease is transmitted by other insects than aphids, and it is quite probable that in addition to grasshoppers it will be found that a number of other insects can act as transmitting agents.

It was found very difficult to detect the mosaic plants in the field because of the masking of symptoms. They were easily distinguished, however, by greenhouse indexing. Leaf roll was usually distinguishable both in the field and by indexing, but under certain field conditions it was very difficult to separate it from severe spindle tuber. The latter disease could usually be detected, however, by the tuber symptoms. Nevertheless, in the final readings for spindle tuber the previous history and the performance of the progeny had to be taken into consideration in order to avoid inaccuracies that would have occurred if the readings for any one year had been considered alone. This difficulty in accurately diagnosing spindle tuber was due, of course, to the number of environmental factors that could seriously affect the intensity of the symptoms.

SUMMARY

Mild and rugose mosaic were transmitted in small amounts in only two of the four years of the experiment, and their spread can be correlated with the occurrence of aphids.

Leaf roll was transmitted slightly more extensively, that is, there was 9.7 per cent infection after four years, but the disease spread only to a distance of about three hills from possible sources of infection. The spread could be correlated with aphid transmission except in 1926, when other insects were probably involved.

⁸ MURPHY, P. A. INVESTIGATIONS ON THE LEAF-ROLL AND MOSAIC DISEASES OF THE POTATO. Ireland Dept. Agr. and Tech. Instr., Jour. 23: 20-34, illus., 1923.

⁹ WERNER, H. O., Op. cit.

Spindle tuber was transmitted more extensively, that is, there was 42.4 per cent after four years. Transmission occurred in all four years, but was more abundant in 1925 and 1927, when aphids were present. The spread of this disease could also be correlated with the presence of grasshoppers, although other insects may have been involved. None of these diseases were spread more than a few rows distant from the sources of infection.

Of the four diseases studied, spindle tuber is undoubtedly the one most to be feared under western conditions, although there was evidence that leaf roll may become a serious problem if it once becomes established in the West.

THE RESPIRATION FACTOR IN THE DETERIORATION OF FRESH VEGETABLES AT ROOM TEMPERATURE¹

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INTRODUCTION

The physiological changes that occur during the rest period of the hardy vegetables have been the subject of many investigations. In general, the chemical composition varies slowly, and it has been found convenient to allow rather large intervals of time to elapse between observations. Among the more perishable vegetables, however, Morse,² working with asparagus, and Appleman,³ and Appleman and Arthur,⁴ with green sweet corn, have found that profound changes in composition occur within a few hours after harvesting, sweet corn, for instance, losing between 50 and 60 per cent of its sugar during 24 hours at 30° C., and very little after that with longer holding; by using a low storage temperature the loss could be very appreciably reduced. In view of the manner in which fresh vegetables are usually handled in markets, stores, and kitchens it becomes important to investigate the changes occurring at room temperature in other green vegetables during the first few hours after they are gathered, and that was the object of the present study. As respiration is probably the principal factor in the deterioration of fresh vegetables at higher temperatures, attention was focused upon that process.

It is recognized that vegetables in markets and stores are subjected to many variables, all of which probably affect the rate of respiration and the rates of the other processes involved in deterioration; there are external variables such as temperature, humidity, and intensity and kind of illumination, and internal factors involving the variety of the vegetable and its degree of maturity. It would be interesting to determine the influence of each of these variables on the rate of respiration, but that was beyond the scope of this study, and so fixed conditions were chosen—a temperature of 30° C., an atmosphere which was saturated with water vapor, absence of light, and that stage of maturity in each case which represents prime eating condition—and the data obtained from the different vegetables under identical conditions should serve as the basis for comparisons.

EXPERIMENTAL PROCEDURE

A diagram of the apparatus used in the experiments is shown in Figure 1. Soda lime in A served to remove CO₂ from the air that entered from out of doors. The reservoir C and the respiration

¹ Received for publication Feb. 11, 1929; issued July, 1929. Published with the permission of the director of the station.

² MORSE, F. W. EXPERIMENTS IN KEEPING ASPARAGUS AFTER CUTTING. Mass. Agr. Expt. Sta. Bul. 172, p. [297]-307. 1917.

³ APPLEMAN, C. O. RESPIRATION AND CATALASE ACTIVITY IN SWEET CORN. Amer. Jour. Bot. 5: 207-219, illus. 1918.

⁴ ——— and ARTHUR, J. M. CARBOHYDRATE METABOLISM IN GREEN SWEET CORN DURING STORAGE AT DIFFERENT TEMPERATURES. Jour. Agr. Research 17: 137-152, illus. 1919.

chamber D were placed beneath the surface of the water in an electrically controlled thermostat. It was assumed that the movement through C would be sufficiently slow to allow the air to assume the temperature of the surrounding bath and become very nearly saturated with water vapor from the layer of water in the bottom of this 2-liter bottle; a dropping funnel (not shown) permitted the addition of more water in C as it was needed. The air was always sufficiently humid to prevent the vegetables from wilting. For the respiration chamber,

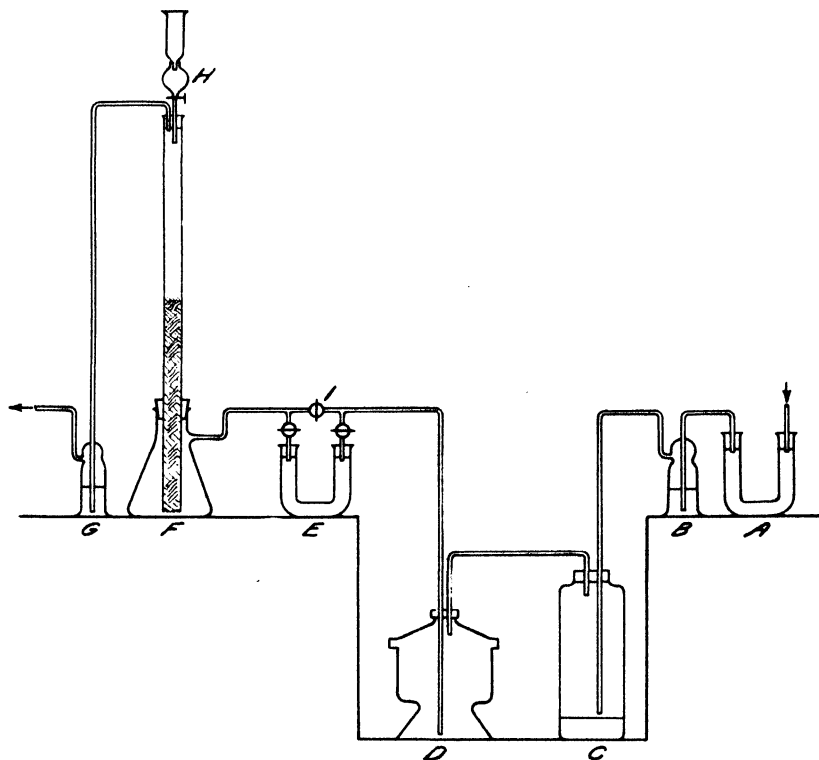


FIGURE 1.—Diagram of the respiration apparatus: A and E contain soda lime; B and G contain a dilute solution of bromothymol blue; C, air reservoir; D, respiration chamber; F, Truog absorption tower; H, 50 c. c. separatory funnel for introducing standard $\text{Ba}(\text{OH})_2$ solution; I, stopcock. C and D are placed in the constant temperature bath

vessels of various sizes and shapes were used, the object being to suit the vessel to the vegetable so as to allow as little waste space as possible—wide-mouthed bottles, small desiccators, and sometimes straight tubes 1-inch in diameter, stoppered at each end, served the purpose. The stopcocks at I permitted the gases to be diverted through the soda-lime tube E between measurements. F is the Truog⁵ absorption tower with the tube lengthened to 30 inches to lessen the danger of loss of solution at the top. $\text{Ba}(\text{OH})_2$ solution was introduced at H. Truog's technic was followed almost exactly in the use of this tower, which proved to be much more efficient than

⁵ TRUOG, E. METHODS FOR THE DETERMINATION OF CARBON DIOXIDE AND A NEW FORM OF ABSORPTION TOWER ADAPTED TO THE TITRIMETRIC METHOD. Jour. Indust. and Engin. Chem. 7: 1045-1049, illus. 1915

any other absorption device tested. At B and G small wash bottles were introduced containing a dilute solution of bromthymol blue. The laboratory distilled water had a pH of approximately 8.0 when CO_2 was removed and consequently gave a good deep-blue color with this indicator. The wash bottles, situated as they were, served to show whether CO_2 entered C or escaped from F. On a few determinations with asparagus (the most rapidly respiring vegetable examined) a small white crucible containing a little of the indicator was placed in the respiration chamber D with the vegetable, and gave assurance by its green tint that the rate of movement of the gas stream was sufficiently rapid to prevent the collection of significant amounts of CO_2 in the chamber. In making determinations the water pump was turned on far enough to draw bubbles almost to the top of F, and the indicator bottles were watched to see that they maintained the proper shade; beyond this no attempts were made to measure or control the rate of flow of gases through the train. Sometimes preliminary experiments were necessary to discover the most convenient amount of any vegetable to use; the weights employed varied from 50 to 500 gm. The respiration chamber was submerged beneath approximately a foot of water, in most cases lead plates covered the top to keep it down, and the thermostat was in a corner of the laboratory far removed from windows, so that the influence of light may be considered negligible.

Experimental material consisted of fresh vegetables of edible maturity brought directly from the garden into the laboratory before the sun was high enough to cause any wilting. All unnecessary bruising was avoided. Vegetables were used for study in the forms in which they are displayed on the market; that is, in the case of beet, green onion, carrot, and lettuce, the whole plant was used; in the case of green beans, pimientos, mangoes, and okra, a very short stem was left attached; tomatoes were pulled off carefully from the vine, and asparagus was cut at the surface of the soil. The choice of vegetables was determined by availability. It should be noted that the temperature in the garden when the vegetables were obtained was usually near 30°C ., so that the material was not subjected to a decided change in temperature when it was placed in the thermostat. The material was washed, dried carefully, and weighed, only perfect specimens being used.

Thirty degrees centigrade was chosen as the working temperature and the bath was held constant to within $\pm 0.5^\circ$. This particular temperature seemed best adapted to the study for two reasons—(1) it was practically the lowest temperature which could be held constant during the summer months with a water thermostat and no special cooling device, and (2) it represents fairly well the average daytime room temperature in this part of the country (Oklahoma).

Determinations were made as follows: After the material for study had been placed in the respiration chamber D, 25 c. c. of 0.25N $\text{Ba}(\text{OH})_2$ was introduced into the funnel H, and the suction of air through the apparatus was begun, the by-pass tube E being used to remove all CO_2 coming from the respiration chamber. When the indicator bottle showed by its color that the CO_2 originally present in the absorption tower had been removed (usually about one-half hour sufficed) the stopcock in H was opened, allowing the $\text{Ba}(\text{OH})_2$ solution to enter the tower. The stopcock of H was closed immedi-

ately and the stopcock I opened, at the same time the two stopcocks on the by-pass tube were closed. The exact time of opening I was noted; the runs were continued for exactly one hour, after which I was again closed, the by-pass stopcocks opened, and the suction reduced. The $\text{Ba}(\text{OH})_2$ solution remaining on the walls of H was washed into the absorption tower F with small amounts of CO_2 -free water. The absorption apparatus was disconnected, the stopper carrying the tube loosened, and the beads washed with CO_2 -free water into the flask, where the excess $\text{Ba}(\text{OH})_2$ was determined by titrating with N/10 HCl in the presence of phenolphthalein. Determinations were repeated at 2-hour intervals throughout the day. The experimental material was kept in the thermostat overnight, but the lid of the respiration chamber was replaced by a loosely arranged wet cloth and the jar was supported with its mouth on a level with the surface of the water, so as to allow free access of air to the vegetables without allowing them to wilt or change temperature. Determinations were continued then through the second day. Blank determinations showed that no measurable amount of CO_2 leaked in or was taken up directly during the manipulations.

EXPERIMENTAL RESULTS

The results, presented in the form of curves, are shown in Figure 2. In every case where there was a significant decline in the rate of respiration during the first 12 hours the whole 30-hour determination was repeated one or more times with fresh material. The curve given in each case was one chosen as typical. Curves on different specimens of the same kind of vegetable were always closely similar in shape and in actual position. The curves appear to approach the logarithmic in form. By mechanically computing the area under the curves for a 24-hour period (from the second until the twenty-sixth hour) the total amount of CO_2 evolved was determined; by assuming that all the carbon lost came from glucose, the weight of this sugar used up may also be calculated. These values are shown in Table 1.

TABLE 1.—Calculations of the carbon and glucose lost per 100 gm. dry weight of various vegetables when held at 30 C° for 26 hours after cutting

Vegetable	Dry matter	Weight of CO_2 evolved between second and twenty-sixth hour	Weight of carbon lost between second and twenty-sixth hour	Calculated weight of glucose lost between second and twenty-sixth hour
	Per cent	Grams	Grams	Grams
Asparagus.....	8.3	20.066	5.473	13.682
Lettuce.....	6.2	9.424	2.570	6.425
Green beans.....	7.7	9.273	2.529	6.322
Okra.....	13.7	7.609	2.001	5.228
Green onion.....	9.3	6.627	1.807	4.518
Carrot.....	13.9	4.659	1.271	3.178
Tomato.....	6.1	3.920	1.069	2.672
Beet.....	9.5	2.958	.807	2.018
Green mango (sweet pepper).....	8.0	2.880	.758	1.962
Red pimiento (short, broad variety).....	9.4	1.893	.516	1.290

* It was only possible to carry the beet determinations over the first day because of a tendency to mold. The values here given were obtained by extrapolation.

No more need be said about the order of the vegetables with respect to the amount of CO_2 evolved than that it is in agreement with the general observation that the most rapidly developing tissue respire

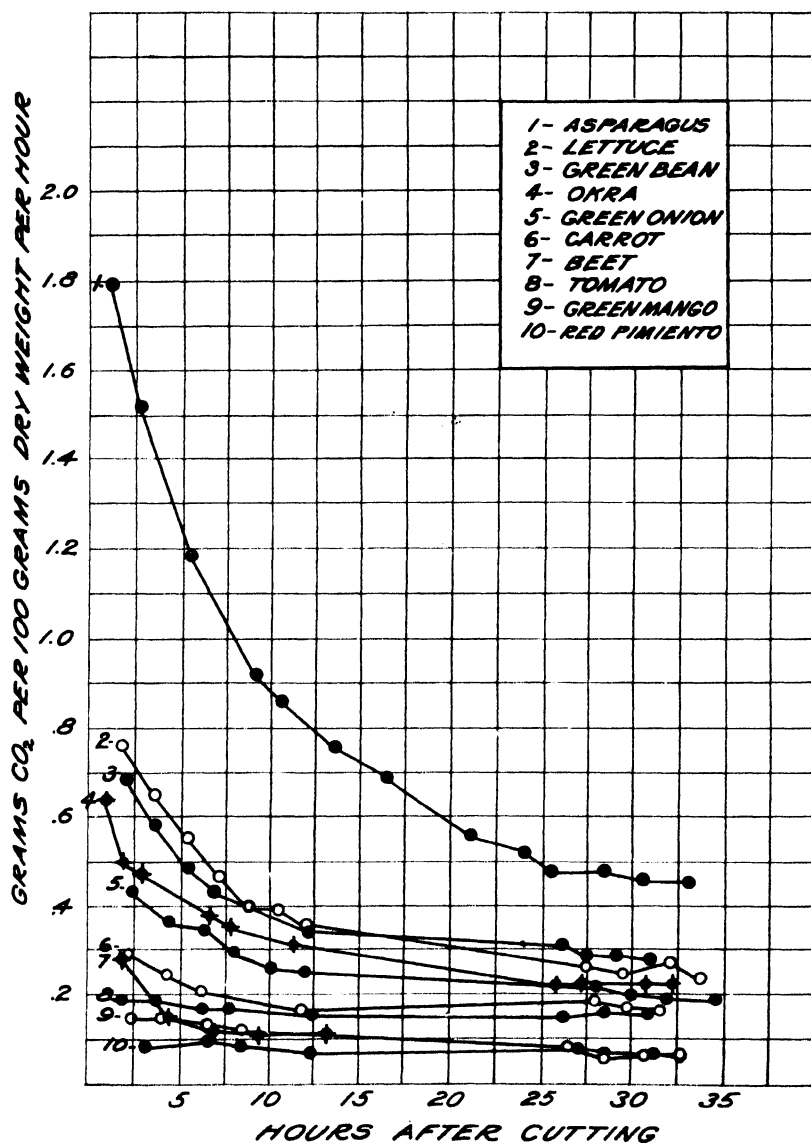


FIGURE 2.—Curves showing decline in respiration rate of fresh vegetables during the first 30 hours after cutting, when held at a constant temperature of 30°C . (± 0.5)

most rapidly. That other significant changes, tending to decrease the food value of the vegetables under consideration, were occurring simultaneously is not to be doubted, such as, for example, an increase

in woody fiber.⁶ This study presents only one phase—although probably the principal one—of the general deterioration process.

The vegetables came out of the thermostat (except the beet) after one and one-half days at 30° C., in an atmosphere nearly saturated with water vapor, looking as fresh and attractive as if they were straight from the garden—there was no outward indication of the changes which had gone on, except that the asparagus had increased a little in length.

SUMMARY

Ten green vegetables of edible maturity were subjected to comparative examination during the first 30 hours after they were harvested. Their changing rates of respiration at 30° C. were measured and plotted, the total amount of CO₂ evolved between the second and the twenty-sixth hours was calculated and the weights of glucose presumably oxidized in producing this quantity of CO₂ were computed. With respect to the amount of gas evolved for equal weights of dry matter during the 24-hour period, the vegetables stand in the following descending order: Asparagus, lettuce, green bean, okra, green onion, carrot, tomato, beet, green mango, red pimiento. The amounts of glucose accounted for vary from 13.682 gm. per hundred grams of dry weight in 24 hours for asparagus to 1.290 gm. for red pimientos.

⁶ BITTING, K. G. DETERIORATION IN ASPARAGUS. Natl. Canners Assoc. Research Bul. 11, 18 p., illus. 1917.



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A PHYSIOLOGICAL STUDY OF ROOTING AND CALLUSING IN APPLE AND WILLOW¹

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INTRODUCTION

In treatises on horticulture but two categories of cuttings are generally recognized—those of softwood and those of hardwood. However, hardwood cuttings may, in turn, be divided into two categories—those that have preformed small but unmistakable root primordia (already present when the cuttings are removed from the original plant) and those that do not possess such primordia. Where they are found, these preformed primordia seem to be simply very small roots or groups of cells that will produce roots upon further growth. Little is known as yet about them or the environmental influences that affect their formation, but they appear to be specific or varietal characteristics of certain plant forms (17).³ The present paper deals only with hardwood cuttings that have such preformed root primordia, apple cuttings (Springdale variety) and willow cuttings (*Salix alba* L.) being used.

Essentially, the rooting of cuttings with primordia consists simply in renewed growth, by which the small dormant roots elongate and push out beyond the bark. In the forms here studied the formation of these roots had occurred long before the cuttings were taken from the trees. These roots may be detected in the bark of 3-year-old and older branches of the Springdale apple, but they have not been observed in the younger wood. Apparently a certain degree of maturity is necessary before they begin to form.

The writer (21) has recently shown that such root primordia are formed by the cambium and apparently only at certain places where branch or leaf-trace parenchyma or primary or secondary rays cross the cambium. The primordia enlarge until the tips reach the outer surface of the bark or project a bit, and then they cease to enlarge and remain dormant until special conditions lead to renewed growth. They are apt to occur in small groups or in groups of 100 or even many more, as well as singly. The raised and modified areas of the bark in and beneath which they lie have long been called burrknots in

¹ Received for publication Mar. 13, 1928; issued July, 1929. This study represents one phase of nursery-stock investigations in the Bureau of Plant Industry of the U. S. Department of Agriculture. The work was done under the official direction of L. C. Corbett, Senior Horticulturist, in charge of the Office of Horticulture.

² For materials and equipment, for much help in planning and conducting the experiments, in interpreting the results, and in presenting them in this form, the writer wishes to express his appreciative thanks to Prof. Burton E. Livingston, Director of the Laboratory of Plant Physiology of the Johns Hopkins University. The writer has also been materially assisted from time to time by Dr. F. W. Haasis, of the same laboratory and of the U. S. Forest Service, and by Mrs. Mildred B. Swingle.

³ Reference is made by number (italic) to "Literature cited," p. 127.

England. The occurrence and formation of root primordia in certain varieties of apple, etc., that exhibit this peculiar phenomenon have been reported by the writer (17, 18, 19, 20).

The occurrence of preformed root primordia under the superficial bark layers in some willows has been demonstrated recently by Van der Lek (11) and by Priestley (14) and was mentioned by Trécul (22) in 1846. In the present studies these have not been given special attention further than to ascertain that they occurred on the willows used in the experiments and to make sure that the roots produced in these experiments were of the type here considered; that is, were actually produced by renewed growth of preformed, dormant roots. In willows, so far as known, there are no burrknots, for here the dormant roots occur only singly or in small groups and do not result in discernible enlargements. It appears, however, that *Salix alba* and the Springdale variety of apple are essentially alike in this particular, that both have preformed roots occurring in the bark of the older twigs. Vöchting (23) reported that root primordia were very much more numerous on stems of willow 2 years old and older than on the most recently formed stems.

ENVIRONMENTAL CONDITIONS NECESSARY FOR ROOTING AND CALLUSING OF CUTTINGS

The production of callus at the ends of cuttings or at lenticels often precedes or accompanies the "pushing" of roots. Callus is a form of tissue hypertrophy, sometimes resulting in large masses of white parenchyma, and it has been thought that there is some definite relation between callus production and root formation. It is still very generally held by propagators that new roots spring from the previously developed callus, in spite of the fact that Corbett (4) long ago pointed out that rooting and callusing are usually distinct processes. It seems probable, nevertheless, that roots may be induced to form from callus under some conditions.

Among the earliest analytical work on the rooting of cuttings were the important studies of Vöchting (23). According to his results with *Salix viminalis* L., gravitation had little or no influence on rooting, while light was found to retard it. His cuttings failed to root in the absence of oxygen.

Boehm (1, 2) stated that the rooting of willow cuttings was greatly retarded or practically prevented when the air about them had a pressure of 3 to 6 atmospheres.

Simon (15) found that callus growth in *Populus nigra* L. was most vigorous with humidities of 85 to 90 per cent. Grau (6) reported that an air humidity of 85 to 95 per cent, with a temperature of 25° C., produced abundant callus but few stem growing points in decapitated cuttings and grafts of tomato and nightshade. With the same temperature, but with air humidity of 60 to 75 per cent, little callus was produced, but many stem growing points were formed. With a maintained air humidity of 76 per cent, she found that the optimum temperature for callus growth was about 28° to 32°, while that for the production of stem growing points was about 22° to 28°.

Curtis (5) obtained results which indicate that rooting was stimulated by proper treatment of the cuttings of *Ligustrum ovalifolium* Hassk. with potassium permanganate, probably through an acceleration of the respiration processes.

Zimmerman (24) reported that willow cuttings failed to root when surrounded by gas containing 90 to 100 per cent of oxygen. The most active rooting in his experiments occurred with a gas mixture of oxygen and nitrogen containing 15 to 33.3 per cent of oxygen. Approximately "normal" growth occurred with 25 per cent of carbon dioxide in the gas about the cuttings, provided the oxygen content was between 25 and 33.3 per cent. According to Zimmerman's experimental results, the optimum temperature for rooting for Late Crawford peaches seemed to be at least as high as 24° C., while the optimum for *Ilex verticillata* (L.) Gray seemed to be at least as high as 27°.

Regarding the influence of the hydrogen-ion concentration of the medium in which cuttings are rooted, apparently the only publications are those of Smith (16) and of Hitchcock and Zimmerman (7). The former worker found that *Coleus* cuttings rooted best in water with a pH value of 7.0 to 7.2, but there was some rooting throughout the range from pH 4.0 to pH 9.2. Inasmuch as acid peat with a pH value of about 4.6 is considered the best practical medium in which to root *Coleus* cuttings, she concluded:

These results indicate that the free admission of air is one of the most favourable aspects of the fibre and suggest that (for this plant at least) given equal supplies of oxygen, a reaction of the medium near neutrality would be preferable.

Hitchcock and Zimmerman (7) presented at the 1926 meeting of the American Society for Horticultural Science some results showing that certain acid-soil plants root better in peat than in sand and that certain others root better in sand than in peat. In the discussion of their paper it was brought out, however, that they had been unable to differentiate between the influences exerted by acidity of the medium and its capacity to supply oxygen and water. It may be that the acidity of the medium as such plays very little part in the rooting of plant cuttings within the ordinary ranges of horticultural experimentation and practice.

Knight (9) has recently reported that the production of callus on cuttings is favored by a higher soil moisture than is required for the production of abundant roots. He does not appear to have distinguished between the influence of high water content as such and its influence in limiting the oxygen supply.

GENERAL NATURE OF THE EXPERIMENTAL STUDIES

The experiments reported in this paper represent an attempt to obtain some additional knowledge, of a somewhat quantitative nature, about the temperature, water, and oxygen relations of root and callus development on cuttings of the Springdale variety of apple and of *Salix alba* L. This study is distinctly of a preliminary nature. It is intended to report the results of a particular group of experiments with particular kinds of cuttings and should not be used as a basis for generalizations about the behavior of plant cuttings in general or even of cuttings with preformed, dormant roots.

Though this paper deals only with the controlling external factors, it is necessary to emphasize the obvious but frequently neglected or slurred generalization that the formation of roots clearly depends not on internal conditions alone nor on external conditions alone but on both categories of influential conditions operating together.

The experimental data were nearly all obtained in the Laboratory of Plant Physiology of the Johns Hopkins University in 1926-27, although some preliminary explorational tests had been made earlier.

METHODS AND PROCEDURES

THE PLANT MATERIAL AND ITS PREPARATION

The apple cuttings used in these experiments were all obtained from one tree of the Springdale variety at the Arlington Experiment Farm, Rosslyn, Va. The willow cuttings were obtained from one tree of *Salix alba* on the grounds of the Johns Hopkins University. Other plant material used from time to time was obtained at the United States Plant Field Station, Glenn Dale, Md. The willow cuttings were usually put into the experiment chambers the day they were removed from the tree. The apple cuttings were put into closed suitcases when removed from the tree and remained there, without the addition of water, for one or two days at a temperature of about 19° C., until they were placed in the chambers in water. In the case of the apple cuttings, as in the case of all other material not placed either in the chambers or in water as soon as removed from the plant, a fresh cut was always made at each end of each cutting.

In each experiment great care was taken to insure like lots of material of each kind for all tests throughout the series. After being made, the cuttings were sorted into as many lots as would be needed, all lots being as nearly alike as possible. The apple cuttings were from wood 3 to 10 years old; most of the willow cuttings were from 2-year-old wood, but some 1-year-old and some 3-year-old wood was used. In most of the experiments 50 cuttings were placed in each chamber, the chambers being 3-liter bottles. In some cases all cuttings in any chamber were of one kind, either apple or willow, but there were generally two or more plant forms represented in each chamber.

No precautions were taken to secure or maintain sterile conditions in the chambers. At the higher temperatures, especially with low rates of oxygen supply, considerable amounts of mold developed, and some fermentation was generally evident at the end of the 10-day experiment period. There were no indications, however, that lack of sterile conditions played any appreciable part in the control of rooting and callusing in any test.

In some of the experiments the cuttings were subjected to a preliminary treatment with water, and in about the same number of experiments they received no preliminary treatment. In most cases the experiment period was either 7 or 10 days, counting from the time the cuttings were made and distributed into groups.

EXPERIMENTAL CONDITIONS

In most cases the cuttings of each test were brought under the special conditions for that test by means of experiment chambers, large-mouth bottles, generally with a capacity of 3 liters, but in some cases larger or smaller. These test bottles were closed with rubber stoppers, suitably provided with perforations in cases where flowing gas was employed, and each bottle contained some water at the bottom. In some of the earlier experiments the bases of the cuttings

rested on wet or merely moist cotton, supported on miniature perforated tables formed from blocks of paraffin, which rested on the bottom of the bottle in each case. It was found, however, that this arrangement was unsatisfactory, since the bases of the cuttings were more or less unequally supplied with water by the cotton. In later experiments the cuttings were in contact with gas only, each one being supported on a wire stilt, applied just before the cutting was placed in the chamber. The stilt consisted of a piece of iron "stove-pipe" wire wrapped once around the cutting near its base and projecting downward about 10 cm. beyond the lower end of the cutting. The free end of the wire rested on the bottom of the test bottle, and the cutting was supported so that no part of it came in contact with the water below.

The experimentation involved different treatments of the cuttings, with respect to (1) temperature, (2) water, and (3) oxygen conditions, which were more or less controlled artificially by special arrangements or procedures. The preliminary nature of the work and lack of time for further refinements made it undesirable or inexpedient to attempt precise controls, except in the case of temperature, which could be readily maintained within narrow limits of fluctuations. The influence of changing temperature was not studied, only continually maintained temperatures being employed.

TEMPERATURE

Most of the experimental results here reported were secured by means of a battery of seven dark chambers with automatically maintained temperatures. These temperature chambers have been described by Livingston and Fawcett (12). The seven chambers are vertical cylinders, each with a covered opening at the top, arranged in a row. Each chamber is surrounded by a water jacket, except above, where there is an insulating cover, and the water is mechanically stirred, which is also true of the air in the chamber. Each chamber is 33 cm. in diameter and 43 cm. deep. One end of the series is electrically heated and controlled; the other is electrically cooled and controlled. For each experiment the controls were set to give a series of seven different maintained temperatures. Temperature fluctuation in any chamber generally did not extend beyond 1° C. above and below the value shown, and fluctuation was usually much smaller. The largest fluctuations were of short duration; they were occasioned by necessary opening of the chambers and did not reach the cuttings in the test bottles.

The test bottles containing the cuttings were in the seven controlled chambers. When flowing gas was employed the tubes leading to and from the bottles entered the chamber through small holes in the top, alongside the removable cover.

Another arrangement for maintaining a single temperature on a very narrow temperature range was employed in some of the experiments. It consisted of a vertical, insulated, cylindrical tank of galvanized sheet iron, 70 cm. high and 50 cm. in diameter, filled with water and provided with a very positive mechanical stirrer operated by an electric motor, and also provided with an electric emersion heater, a mercury thermoregulator, and suitable relays. The test bottles containing the cuttings were suspended in the rapidly circulating water of the tank. No means was provided for cooling this tank,

and it was consequently used only for the maintenance of temperatures above the maximum one of the greenhouse room in which the apparatus was located.

A few experiments were carried out in the dark room of the laboratory, which provided a temperature generally about 19° C., with fluctuations between 18° and 22°.

MOISTURE

The moisture and conditions of the gas about the cuttings were those of approximate saturation for the temperature employed. As has been said, each test bottle had some water at the bottom. When flowing gas was employed the gas stream was first passed through water in a humidifying bottle, so that the gas entering the test bottle was practically saturated with water vapor. Upon entering, the gas bubbled through the standing water at the bottom of the bottle, and complete saturation was thus very nearly approached.

In some of the experiments the cuttings were inserted in the test bottles without any special preliminary treatment. Doubtless these cuttings had lost a little of their original water content, since they had been removed from the tree for a considerable period and had been brought into the warmer laboratory room from the cooler out of doors.

In some experiments the influence of preliminary wetting of the cuttings was studied. This preliminary treatment was applied just before the cuttings were placed in the test bottles, the aim being to increase more or less the water content of the cuttings, either through-out or near the cut ends or near the base alone. Several different preliminary treatments with water were employed, as shown just below and as summarized in the tabulated results. The time consumed in the preliminary treatment was, in every case, included in the 10-day period of the experiment.

(1) Cuttings were completely injected by being submerged in water, with the air above first nearly exhausted for 15 minutes and then allowed to reenter the injection chamber. They remained in the water 22 hours after atmospheric pressure had been resumed. (Experiment 8.)

(2) Cuttings were completely submerged in water for one hour without injection. (Experiment 14.)

(3) The bases of cuttings were submerged in water for 19, 22, 24, or 48 hours without injection. (Experiments 10, 9, 15, 16, respectively.)

(4) The bases only were injected (as in 1, above), and the bases remained under water for 19 hours after atmospheric pressure had been removed. (Experiments 11, 12.)

OXYGEN

In many of the experiments gas flowed through the test bottles throughout the experiment. The gas stream was passed through the bottle by means of suitable tubes borne by a 2-hole rubber stopper set in the bottle mouth. The rate of flow was maintained by means of constant pressure and properly adjusted orifice resistance, a cotton-kaolin orifice control like that devised by Hutchins (8) being used.

Constant pressure was maintained by means of a mercury pressure regulator with 4 cm. of mercury column and continuous escape of some gas. Several rates of gas flow were employed, approximately as follows:

300 c. c. per hour (experiments 1 to 5 and experiment 7, apple and willow).

50 c. c. per hour (experiment 6, apple and willow).

200 c. c. per hour (experiments 8 to 16, apple, and experiments 8 to 12, willow).

Flowing air was employed in experiments 2 to 4 and 8 to 12 (apple and willow) and in experiments 13 to 16 (apple). Flowing oxygen was employed in experiments 1 and 7 (apple and willow), and an artificially prepared mixture of oxygen and nitrogen was employed in experiments 5 and 6 (apple and willow), the mixture containing 5 per cent oxygen. Glass and lead tubing were used, with a minimum amount of rubber tubing for connections. When flowing air was used the requisite pressure gradient was maintained by means of an ordinary water aspirator pump attached to the laboratory water supply, the mercury pressure regulator and the orifice controls being suitably arranged. Air entered each test bottle from the maintained-temperature chamber in which the bottle stood, the air being thus at the proper temperature in each case, and it was discharged throughout the aspirator. When flowing oxygen or the artificial mixture of oxygen and nitrogen was used it was drawn from an ordinary commercial tank by means of a control valve, no pump being required.

The oxygen-nitrogen mixture was prepared in a commercial gas tank at high pressure, the proportions of the two gases being determined by their partial pressures in the mixture. The method used for preparing this mixture was that employed by Prof. E. K. Marshall, of the Johns Hopkins Medical School, who brought it to the writer's attention. In one case, for example, an empty tank was connected to a tank of nitrogen at an initial pressure of 1,800 pounds per square inch, the connection being made by means of a piece of suitable copper tubing 75 cm. long with a mechanical pressure gauge inserted in the middle and with a suitable coupling at each end. After the two tanks had been connected their valves were opened, and the pressure was allowed to become about the same in both. Both valves were then closed, and one tank was removed from the connecting tube and replaced by a tank of oxygen at a pressure of 1,200 pounds per square inch. After an hour, during which the nitrogen (cooled by this expansion) again assumed the temperature of the surroundings, the valve on the nitrogen tank was opened (the valve on the oxygen tank remaining closed), and the pressure of the nitrogen was read from the gauge; it was 910 pounds per square inch. Then the oxygen valve was opened, and oxygen was allowed to pass into the nitrogen in several installments till the gauge reading, with the oxygen valve again closed and after a suitable period for temperature recovery, was 958 pounds per square inch. Then both valves were closed, and the coupling tube was removed. The tank that had been originally empty now contained a mixture of nitrogen and oxygen, with partial pressures of 910 and 48 pounds per square inch, respectively; i. e., a mixture of approximately 95 per cent of oxygen. This method of preparing the gas mixture does not involve differential solubilities of the gases in a liquid, as when gasometers are used, and it

provides the mixture conveniently in a pressure tank, under pressure suitable for long runs of such experiments as are here considered. The limits of precision are set by the precision of the gauge readings.

Commercial nitrogen and oxygen such as were used in these studies are prepared by the distillation of liquid air and may be supposed to contain some impurities, which are limited to the constituents of air. The nitrogen contains a very little oxygen (perhaps 0.5 of 1 per cent), and the oxygen contains a corresponding amount of nitrogen. Carbon dioxide and argon are present in such small quantities that they may be ignored. Since tests of similar gases by other workers indicate no other impurities as likely to be present, no scrubbing arrangements were employed, and no analyses were made.

When only small quantities of gas mixtures were needed, other and less precise methods of mixing were employed. The ordinary procedure with a water gasometer was followed in some cases. In some of the preliminary experiments without maintained temperatures and without flowing gases, rubber bladders were used, the gas within the test bottle being thus maintained under slightly more than atmospheric pressure. With this arrangement, which was similar to that used by Free,⁴ only nitrogen, oxygen, or carbon dioxide was used with any given bottle and bladder, and mixtures were not attempted. In a few experiments a method described by Cannon (3) was employed. The oxygen of the air in an inverted test tube was practically all removed very rapidly by means of moist, bright steel wool in the closed end of the tube, the cuttings being initially placed in the tube and the lower end of the latter being closed with agar gel.

DURATION OF THE TESTS

It has been made clear by the work of Lehenbauer (10) on maize seedlings that the temperature graph for any form of plant growth may be expected to vary considerably with the length of the experiment period, especially with regard to the cardinal temperatures. If the period is very short, no growth is to be detected except at temperatures within or in the vicinity of the optimum range. This generalization probably applies to the study of the rooting and callusing of cuttings. A 10-day period was arbitrarily adopted for most of these tests, simply because this period proved long enough to give apparently satisfactory and consistent results. A 7-day period was used for some of the experiments with willow, especially those carried out in the single-chamber apparatus described above, maintained only at optimal or supra-optimal temperature.

OBSERVATIONS AND RECORDS

At the end of the 10-day period the cuttings were all removed from the test bottles and records were made of the amount of rooting and callusing that had occurred. The data thus secured were so averaged as to give a numerical index representing each form of activity at each temperature of an experiment. For callusing, observations were made on base and top callus separately.

⁴ FREE, E. E. THE OXYGEN REQUIREMENT OF PLANT ROOTS IN RELATION TO SOIL AERATION. (Diss. Johns Hopkins.) 1917. [Unpublished.] (See also reference 15, p. 128.)

EXPERIMENTS WITH APPLE CUTTINGS

The data from the experiments on rooting and callusing of apple cuttings in flowing gas and with maintained temperatures are presented in Table 1. For each temperature used (except the highest in each experiment) the tabulation shows an index value representing the "pushing" of roots and two values for the formation of callus, the first for the tops and the second for the bases of the cuttings, which were all placed with their basal ends downward. For the highest temperature value in each series no rooting or callusing values are given, since no activity of either sort was observed at these temperatures. Consequently, the temperature shown at the extreme right in each line of the table may be taken to represent either the temperature maximum for all three kinds of activity in the experiment in question or else a value somewhat higher than the maximum in each case.

The values given for pushing of roots represent in each instance the proportion of the cuttings used that showed more or less pushing of roots at the end of the 10-day period. These are calculated on the basis of 10; the value 10 indicates that every cutting showed some pushing of roots, the value 6 indicates that six-tenths of the cuttings showed this activity, and so on. These rooting values give no indication of the number of protruding roots observed or the lengths of the roots. If a cutting showed but a single slightly protruding root it was recorded as pushing, and the record was the same if there were several protruding roots, some of which were 1 cm. or more in length. It would doubtless have been more to the point if the number of root primordia had been determined for each cutting together with the number and length of the protruding roots at the time of observation, so that the index of pushing might have been based on the total number of primordia available instead of on the number of cuttings used, while the amount of growth after the roots emerged might have been shown quantitatively in terms of their elongation. In the case of the willow cuttings, however, the counting of primordia would not have been possible for these are not visible.

The amount of callus developed at the top and at the base of the cuttings during the 10-day period is shown in the table by relative numerical values between 0 (no callus observed) and 10 (the maximum amount of callus observed in this entire study). The amount of callus formed per cutting was not measured precisely, and these data are based on a somewhat rough scoring method that was developed during the progress of the studies. The score value 10 indicates that the region of the cutting considered (top or base) was about half covered with thick, apparently vigorous callus.

Besides the experiments with both flowing gas and seven different maintained temperatures, several experiments were carried out with other treatments. The results of some of these additional experiments are given hereinafter in Tables 3, 4, and 5. Table 3 presents the numerical results of experiments 17, 18, and 19, with maintained temperatures but without flowing gas. These results are arranged like those in Table 1 except that values for lenticel callus-formation are here included. The latter values are based on a scale of 10 like those for top and base callus formation. Table 4 gives the rooting score values from experiments 20 to 23, with different treatments, conducted in a series of four chambers and in the laboratory dark room. In two other dark-room experiments, sealed chambers of different capacities were used, the chambers of experiment 24 being filled with air and those of experiment 25 being filled with oxygen at the start. The numerical values for rooting and callusing from these experiments are given in Table 5, on a scale of 10, just as in Tables 1, 2, and 3.

The data given in the tables will be generally discussed with reference to temperature graphs that have been drawn to represent each series.

ROOTING OF APPLE CUTTINGS

EXPERIMENTS WITH FLOWING GAS AND SEVEN DIFFERENT MAINTAINED TEMPERATURES

This section deals with the data given in Table 1 and with the corresponding graphs. (In all the charts, figs. 1 to 17, the numbers

on the ordinate indicate relative degrees of rooting or callusing on a scale of 10.) Experiment 1, started November 8, 1926, gave what appears to be the usual form of temperature curve for growth, with minimum about 8° C. or higher, maximum about 39° or lower, and optimum about 28°.

(Fig. 1.) These cuttings were well supplied with water-saturated oxygen, and the bases were not covered by water, for the cotton on which they rested was not nearly saturated. It is possible, however, that some water was absorbed from the damp cotton. Experiment 2 (fig. 1), started November 23, 1926, was like experiment 1, except that air instead of oxygen was continually supplied. The temperature maximum here is indicated as below 41°, and it may have been about 39° or lower, as in experiment 1. The optimum for experiment 2 is not so clearly shown as that for experiment 1, but it may have been about the same temperature as that for experiment 1 or somewhat higher. Experiment 2 differed from experiment 1, however, with respect to the minimum, which appears to have been above 18°, perhaps even about 20°. No explanation of this difference is apparent, for it seems hardly probable that the lower oxygen supply of the second series could have retarded

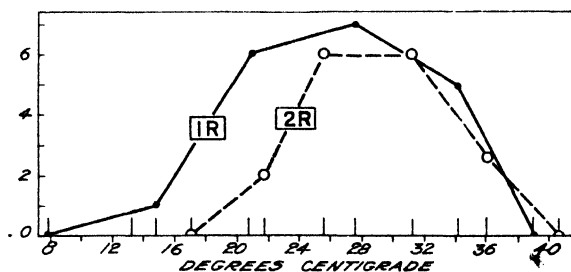


FIGURE 1.—Rooting of apple cuttings in closed chambers: 1R, in flowing oxygen; 2R, in flowing air

root pushing for the lower temperatures without showing a still more marked retardation for the higher ones. The dates are nearly the same for the two experiments, and seasonal differences can hardly be considered as explaining the observed differences.

Experiments 3 and 4 (fig. 2) were planned to compare the behavior of cuttings with bases surrounded by wet cotton with that of other cuttings with bases in water-saturated air, both series being otherwise alike, continually supplied with flowing air. It appears that the wet cotton wrappings at the bases of the cuttings retarded the pushing of the roots (fig. 2, 3R), for no roots emerged except at temperatures about 30° and 35° C.; furthermore, emerging roots were here confined to the upper half of the cuttings, and the amount of root growth was noted as very slight. The minimum in this case seems to have been above 26°, the maximum below 40°, and the optimum perhaps about 34° or 35°.

In experiment 4, however, with no water films about the bases, the growth curve indicated is of the usual form, with minimum about 18° and maximum below 40°, while the optimum is indicated in this case as about 30°, though it may have been lower. This graph for experiment 4 is similar to that for experiment 2, which had similar conditions. It is remarkable that the more plentiful oxygen supply of experiment 1 seems to have accelerated the pushing of roots at the lower temperatures, giving a much lower apparent minimum than that shown for experiments 2 and 4. If it be supposed that the wet cotton of experiment 3 acted to retard the access of oxygen to the cuttings, then it may be significant that this wet-cotton treatment appears, when compared with experiment 1, to have produced a retardation similar to that of experiments 2 and 4 but still more pronounced.

In Figure 2 are presented this comparison between the data for experiments 2 and 4 (considered as alike) on the one hand and experiment 13 on the other, which indicates a pronounced difference that may be related to the seasons of the year at which the experiments in question were begun. The lower graph of the upper part (2, 4R) represents the average of experiments 2 and 4 (November 23 and December 7), and the upper graph (13R) represents experiment 13 (February 26). With respect to external conditions these two graphs represent no considerable differences, and the marked difference shown seems to have been due to changes that had occurred in the twigs themselves between November and March. It appears that the optimum temperature was definitely lower (about 24° C.) and that the graph was much flatter at the top for the later experiment, and also that the minimum was much lower (probably below 10°

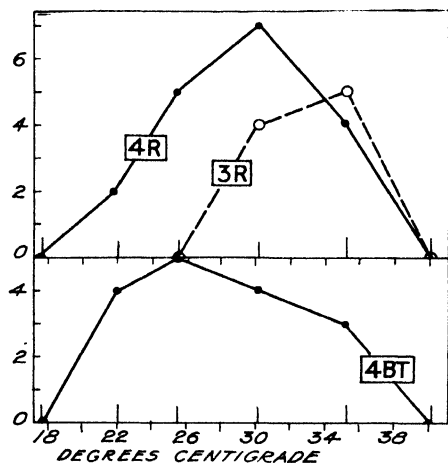


FIGURE 2.—Rooting (3R and 4R) and base-callus and top-callus formation (4BT) of apple cuttings in closed chambers with flowing air; 3R, bases of the cuttings resting on wet cotton; 4R, bases resting on wire stilts

and perhaps as low as 9°). The maximum temperature appears to be about the same for both seasons (about 40°).

Experiments 5 and 6 (fig. 4) were characterized by a low oxygen content in the flowing gas (5 per cent of oxygen and 95 per cent of nitrogen) and were planned to bring out a comparison between rapid and slow flow, the rate of flow for the gas mixture being only one-sixth as rapid for experiment 6 as for experiment 5. The oxygen supply for experiment 5 was lower than that for experiments 2, 3, and 4 (with flowing air) and much lower than that for experiment 1 (with flowing oxygen).

The graphs of experiments 5 and 6 agree very closely. Apparently these two sets of conditions were too nearly alike to bring out significant differences in the pushing of the roots. Only with respect to the maximum temperature do these graphs disagree, and it is to be noted that, although no pushing was observed at about 33° C. in experiment 6, yet the amount of pushing for this temperature in the other experiment was very small. If the two series are considered together, the minimum may be estimated as above 14° , the maximum as about 34° , and the optimum as about 24° to 26° . Apparently the low concentration of oxygen

here acted chiefly to reduce the index values for the supra-optimal temperatures and to shift the maximum toward the left.

Experiment 7 (fig. 3, lower part; fig. 5, upper part) resembled experiment 1, but the cuttings of the later series were entirely surrounded by moist gas (being supported on wire stilts) while those of the earlier series rested on damp cotton. The two experiments agree in respect to the cardinal temperatures (about 8° , 28° , 39° C.), but the values for rooting are higher for experiment 1 in the intermediate suboptimal temperature range. It is not easy to relate this difference to the seasonal difference between these two series, for it is in the opposite direction from that shown between experiments 2 and 4 on the one hand and experiment 13 on the other. Experiments 2, 4, and 13 were with flowing air, while experiments 1 and 7 were with flowing oxygen, but this circumstance can hardly give a clue to an explanation of the observed difference between the results of the two

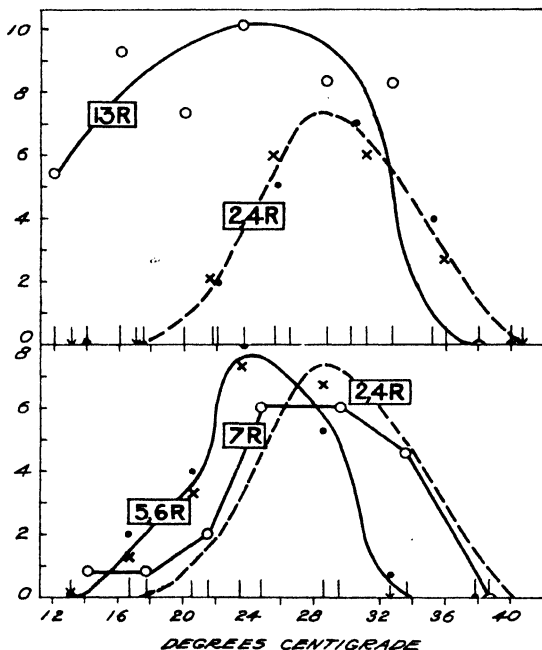


FIGURE 3.—Upper portion: Seasonal differences in the rooting of apple cuttings in closed chambers with flowing air. Lower portion: Rooting of apple cuttings in closed chambers with different flowing gases, experiments 2 and 4, air; 5 and 6, 5 per cent oxygen in nitrogen; 7, oxygen

series. It is suggested that the difference between the graphs for experiments 1 and 7 may be related to water supply, for the cuttings of the earlier series rested on damp cotton, whereas those of the later series were entirely surrounded by moist gas, as has been noted. However, the remarkable similarity of the graphs for experiments 2 and 4

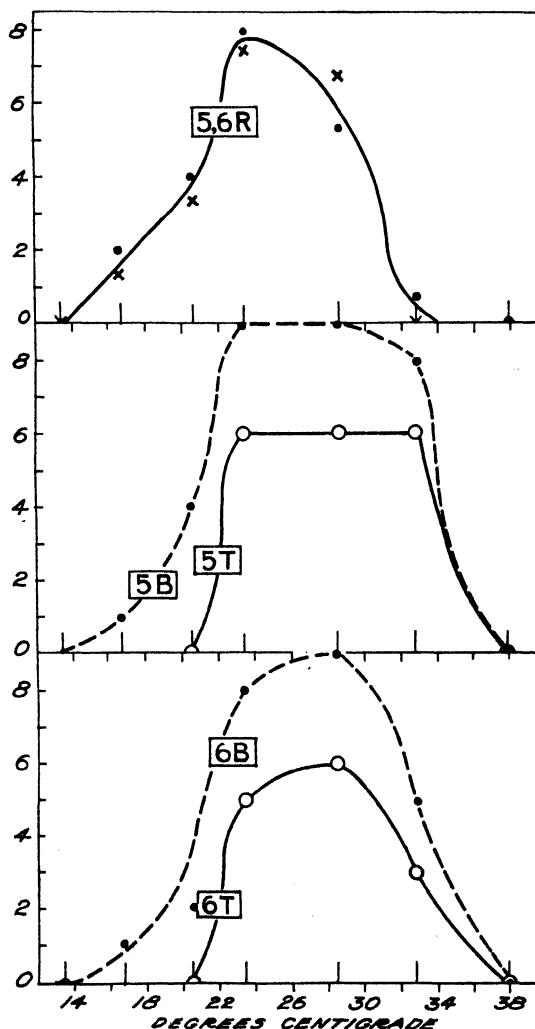


FIGURE 4.—Rooting (5, 6 R), top-callus formation (5T, 6T), and base-callus formation (5B, 6B) of apple cuttings in closed chambers in a flowing-gas mixture of 5 per cent oxygen in nitrogen. Experiment 5, 300 c. c. gas per hour; experiment 6, 50 c. c. per hour

The graph for experiment 9 has a very flat-topped form, indicating a broad range of approximately optimal temperatures. An optimum temperature in this case can not be estimated; it is represented by a range from about 14° to 34° C. No minimum temperature was

render such an explanation at least doubtful, unless the difference between flowing air and flowing oxygen is considered as influential in this respect, for the cuttings of experiment 2 had the same moisture treatment as those of experiment 1, and the cuttings of experiment 4 had the same moisture treatment as those of experiment 7. The difference between the graphs for experiments 1 and 7 were related to unknown conditions.

Experiments 8 and 9 (fig. 6, upper part) were alike except for preliminary water treatment, both having flowing air. For experiment 8 the cuttings had been first injected and then completely submerged in water for 22 hours. For experiment 9 they had not been injected, and only the bases had been submerged during a soaking period of 22 hours. It is clear that the more abundant water treatment in experiment 8 greatly retarded the pushing of roots and completely inhibited it for temperatures above about 24° C.

reached, but it must be estimated as probably as low as 8° or 9° , and the maximum was about 39° .

The exceptionally broad optimal range shown by the values from experiment 9 suggests that some special preliminary water treatment may be regarded as beneficial, although it must not be too extensive. Experiments 10, 11, and 12 (fig. 7, upper part), the last two being duplicates, gave graphs essentially much like that for experiment 9 and appear to support this suggestion. All had preliminary water treatment, and the optimal range seems to have extended from about 16° to about 29° C., if the results of the three tests are combined. Soaking alone (experiment 10) gave the broader optimal range, while an injection at the beginning of soaking seems to have narrowed this range somewhat at both ends. Experiments 9, 10, 11, and 12 appear to show that rooting in February was not much influenced by temperature differences between about 16° and 33° when some extra water was added to the tissues by preliminary water treatment. But the data from experiment 8 indicate, as has been said, that such preliminary water treatment may be excessive, resulting in a pronounced retardation and even in a complete inhibition of the pushing of roots. It may be that the internal water supply was somewhat inadequate in the cuttings used at that time, unless preliminary water treatment had been given. The retarding action of excessive preliminary treatment with water may have been due to a water plugging of air spaces in the tissues of the cuttings, this plugging perhaps operating to retard the supply of oxygen to the root primordia.

The growth values for the optimal temperatures were very high (9) in these experiments, as well as in experiment 9. As to the indications regarding the minimum and maximum temperatures, experiments 9, 10, 11, and 12 suggest a low minimum, hardly above 8° or 9° C., while they agree very well in indicating a maximum of about 38° to 39° .

Experiments 13, 14, 15, and 16 (figs. 8 and 9) were all carried out at the same time, beginning February 26, 1927, and all had the same gas treatment (flowing air). They were planned to throw more light on the relation of root pushing to preliminary water treatment. The cuttings of experiment 13 were without such treatment, while those of the remaining tests received different water treatments. If the graph of experiment 13 is employed as a standard for comparison, the graphs of Figure 8 indicate that the optimal range was markedly widened by completely soaking the cuttings one hour at the start of the test. This range and the form of the temperature graph were

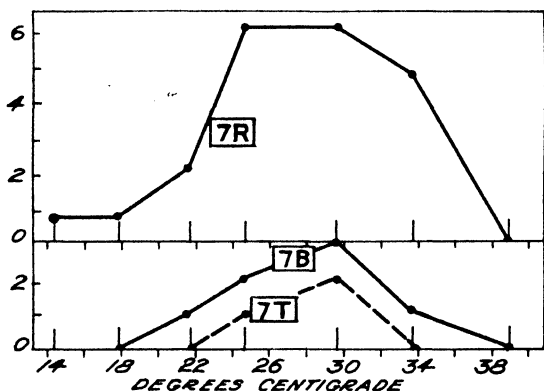


FIGURE 5.--Rooting (7R), top-callus formation (7T), and base-callus formation (7B) of apple cuttings in closed chambers with flowing oxygen

not markedly altered from those shown for experiment 13 by a preliminary soaking of the bases of the cuttings for 24 hours (experiment 15), and the range was much shortened and the graph was also much depressed (for the supra-optimal temperatures) when the bases were soaked for a preliminary period of 48 hours (experiment 16).

It appears that these cuttings, made February 26, were greatly improved with regard to rooting at suboptimal and supra-optimal temperatures (with flowing, water-saturated air) if they had been given the preliminary water treatment of experiment 14, that they were not seriously affected by the treatment of experiment 15, but that the water treatment of experiment 16 was markedly excessive (with consequent retardation) for temperatures above

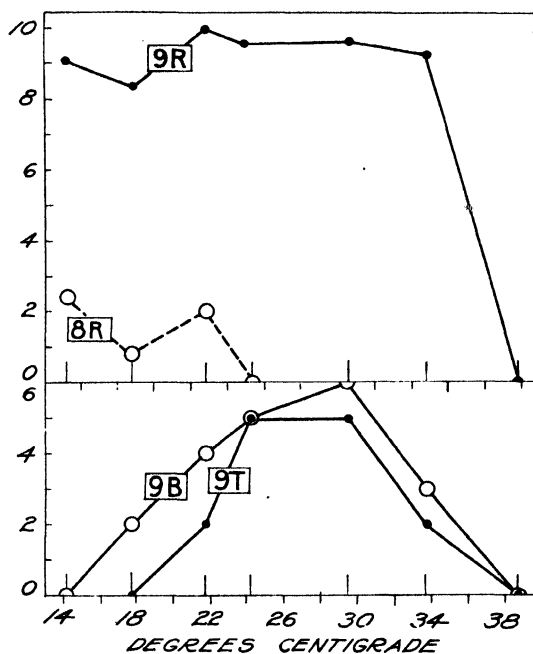


FIGURE 6.—Rooting (8R, 9R), top-callus formation (9T), and base-callus formation (9B) of apple cuttings in closed chambers with flowing air. The cuttings of experiment 8 were injected with water and remained completely immersed for 22 hours, while those of experiment 9 were soaked in water (bases only) for same length of time

10, 11, and 12, in showing very high values (10) for the indices of rooting activity. They also agree in indicating low minimal temperature values (below 8° C. for experiment 14) and in indicating a temperature maximum about 38° (except for experiment 16, with excessive preliminary water treatment, for which the temperature maximum appears to have been in the vicinity of 33°).

In addition to the numerical data taken from line 15 of Table 1, and represented by the upper line of the upper portion of Figure 10, the lower line (4r) of the same figure shows an estimate of the actual temperature curve of root pushing based upon additional observations made at the completion of experiment 14. It was noted that the largest number of roots per cutting and the greatest growth in length

for temperatures above about 26° C. These tests seem to strengthen the suggestion derived from experiments 8 to 12. When the soaking at the bases of the cuttings was limited to a period of 24 hours, it appears that the favorable influence of preliminary soaking (perhaps due to a resulting increased water content of the cuttings) nearly offset a concomitant unfavorable influence (perhaps related to decreased oxygen supply), but that when soaking was prolonged to 48 hours the unfavorable influence greatly predominated, especially for supra-optimal temperatures.

The results of these last four experiments agree in general with those of experiments 9,

occurred at about 23.7° C. and at about 28.7°. At these temperatures almost every available primordium was observed to be pushing, and roots 2 or 3 cm. in length were evident in many cases. The same amount of root growth was evident for 23.7° and for 28.7°, but roots were fewer and shorter at a temperature of 32.8° and at temperatures of 20.3° and lower; however, as has been stated, some root growth was observed on nearly every cutting. Similar qualitative estimates on number of roots per cutting and average root length were made at the completion of experiment 9 (bases soaked 22 hours) and experiments 10, 11, and 12 (bases soaked 19 hours). In all of these five experiments it was observed that the amount of growth in length as well as the proportion of available primordia that had pushed seemed to be the same at about 24° and about 29°.

Although no cuttings were tested at temperatures below 12° C. in experiment 14, the graph for this experiment (fig. 10, 14r) is extended to the zero line at 9°, for while every cutting subjected to a temperature of about 12° showed some root activity, such activity was so slight as to indicate that the zero point for discernible root growth within 10 days was not far from 9°.

It is possible from these 16 experiments to assign 41° C. as the upper temperature limit at and beyond which no root pushing took place, but no definite minimum is apparent. Although the number of roots per cutting was small and the growth in length was very slight, nevertheless a very slow growth rather than the complete stopping of growth seemed to be indicated for the lowest temperatures tested. In fact, subsequent results showed that the absolute minimum temperature for pushing of roots was below 4.5°. Branches from 1 to 2.5 meters long were cut from the tree on February 23 and were placed with their bases in water, and when these were examined May 14 they showed a very slight amount of pushing by almost every avail-

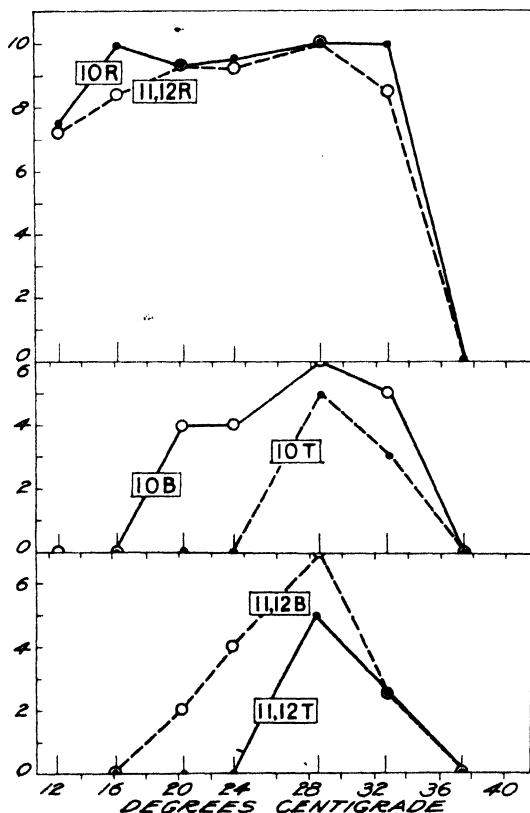


FIGURE 7.—Rooting (10R, 11, 12R), top-callus formation (10T, 11, 12T), and base-callus formation (10B, 11, 12B) of apple cuttings in closed chambers with flowing air. Experiment 10, bases of cuttings soaked 19 hours; experiments 11 and 12, bases injected with water and soaked 19 hours

able primordium except those actually in the water. Nevertheless, growth of buds seemed to have been entirely stopped at this temperature.

A comparison of the graph of experiment 13 with the corresponding one of experiments 2 and 4 (see fig. 3) indicates, as has been noted,

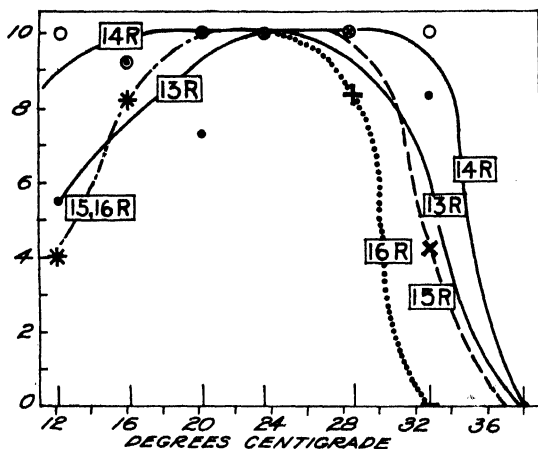


FIGURE 8.—Rooting of apple cuttings in closed chambers with flowing air and with different water treatments: Experiment 13, no water treatment; experiment 14, material completely soaked 1 hour; experiment 15, bases soaked 24 hours; experiment 16, bases soaked 48 hours

30° to about 24°, (2) in shifting the minimum temperature in a similar manner (from about 18° to 8° or 9°, or lower), and (3) in markedly raising the index values for all temperatures below the supra-optimal temperatures.

SUMMARY OF RESULTS OF EXPERIMENTS 1 TO 16

The main numerical results of the several series are brought together for convenient comparison in Table 2, which is arranged to bring out the relations with regard to the degree of rooting for maintained temperatures near the optimum and with regard to the water treatment.

The later series, beginning on or after February 3, all gave very high values for the index of rooting (9 or 10) for the optimum temperature, with the single exception of experiment 8, the cuttings of which apparently had had excessive preliminary treatment with water. None of the earlier series gave index values higher than 8, no matter what the treatment. On the other hand, none of the earlier series gave these values lower than 6, except when the bases of the cuttings rested on wet cotton (experiment 3, with index value of 5). While this evidence is not very pronounced, it suggests that these cuttings had a somewhat greater tendency to root, under the range of conditions tested, in the latter part of the season than they had in the earlier part.

that the results of the later series differed from those of the earlier ones by showing a much greater rooting activity for the maintained temperatures below about 30° C. This apparent seasonal relation (perhaps due to differences in what may be termed the physiological maturity of the root primordia and their surrounding tissues) seems to have resulted (1) in shifting the later optimal temperature (experiment 13) downward on the temperature scale from about

TABLE 2.—Summary of results of experiments 1 to 16 in rooting and callusing of apple cuttings

Experiment number	Date started	Flowing gas	Water conditions	Data for rooting			Data for top callus			Data for base callus		
				Approximate cardinal temperatures		Index for optimum temperature	Approximate cardinal temperatures		Index for optimum temperature	Approximate cardinal temperatures		Index for optimum temperature
				Mini- mum	Opti- mum		Mini- mum	Opti- mum		Mini- mum	Opti- mum	
1	1926 Nov. 8	Water saturated O ₂	Bases on damp cotton.....	° C. 5	° C. 28	° C. 39	° C. 7	° C. 39	° C. (a)	° C. (a)	° C. (a)	(a)
7	1927 Jan. 21do.....	Cuttings not in contact with liquid water.	8(?)	28	39	6	22	30	34	2	3
5	Jan. 5	Water-saturated mixture:do.....	14	24	34	8	20	24-35	38	6	9
6do.....	5 per cent O ₂do.....	14	24	34	7	20	29	38	6	9
4	1926 Dec. 7	Water-saturated air.....do.....	18	30	40	7	18	26	40	5	5
13	1927 Feb. 26do.....do.....	9(?)	24	38	10	16	24-29	38	6	9
2	1926 Nov. 23do.....	Bases on damp cotton.....	18	25	40	6	(a)	(a)	(a)	(a)	(a)
10	1927 Feb. 26do.....	Completely soaked 1 hour.....	8(?)	14-32	38	10	16	29	33	8	9
14	Feb. 15do.....	Bases soaked 10 hours.....	8(?)	16-33	38	10	23	29	38	5	6
9	Feb. 3do.....	Bases soaked 22 hours.....	8(?)	14-34	38	10	18	24-30	39	5	6
15	Feb. 26do.....	Bases soaked 24 hours.....	9(?)	20-31	38	10	16	29	33	5	3
16do.....do.....	Bases soaked 48 hours.....	9(?)	21-24	33	10	24	29	33	5	2
11, 12	Feb. 15do.....	Bases injected and soaked 19 hours.....	9(?)	20-29	38	10	23	29	38	5	7
3	1926 Dec. 7do.....	Bases on wet cotton.....	26	35	40	5	26	30	35	2	(b)
8	1927 Feb. 3do.....	Injected and completely soaked 22 hours.....	8(?)	14-22	24	2	(b)	(b)	(b)	(b)	(b)

* No observation.

* No callus.

It is also apparent from Table 2 that preliminary water treatment tended to lower the temperature minimum and to extend the optimal temperature range. Although it can not be definitely stated from these preliminary data, it seems to be indicated that the temperature minimum and the optimal temperature range were somehow partially determined by the water relations, being also influenced, perhaps, by internal conditions that were innate in the cuttings themselves at the beginning of a test, conditions connoted by the vague expression "maturity of tissues." The relative intensity of rooting, mentioned

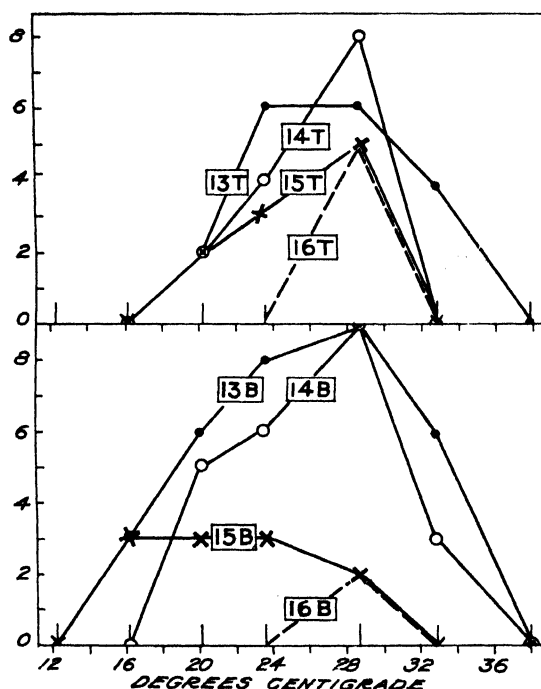


FIGURE 9.—Top-callus formation (13T, 14T, 15T, and 16T) and base-callus formation (13B, 14B, 15B, and 16B) of apple cuttings in closed chambers with flowing air and with different water treatments: Experiment 13, no water treatment; experiment 14, material completely soaked 1 hour; experiment 15, bases soaked 24 hours; experiment 16, bases soaked 48 hours

curve of rooting at optimal and supra-optimal temperatures without materially affecting the curve at sub-optimal temperatures. Further effects of low supplies of oxygen, especially at the higher temperatures, were apparent in experiments 8, 10, 15, and 16, where probably a considerable part of the residual air of the cuttings had been replaced by water. In such cases it seems that the oxygen supply and the temperature were very closely related; the higher the temperature the greater the supply of oxygen required for optimum growth.

As is shown by the graphs, in none of the experiments in which gas was flowing through the chambers did it seem to be indicated that large amounts or high pressures of oxygen had exerted any retardation upon the rooting of the apple cuttings. On the other

just above, seems to have been influenced by maturity. The optimum temperature for rooting can not be fixed from the data at hand, though it is probably safe to guess that it lay generally in the vicinity of 26° to 28° C., except in some cases where special treatment had been applied to the cuttings. With the exception of excessively wet cuttings and those in experiments 5 and 6, which were subjected to low concentrations of oxygen, it seems that the maximum temperature was about 39°, and this did not appear to alter with maturity or with the season of the year.

Independently of any effect brought about by water content, experiments 5 and 6 show that low supplies of oxygen may change the

hand, the use of flowing oxygen did not seem to show any particular advantage over flowing air in the rooting of these cuttings. Thus from these experiments the use of partial pressures of oxygen higher than that in air with a total pressure of 1 atmosphere does not seem to be desirable.

CALLUSING OF APPLE CUTTINGS

In spite of the conclusion that callusing is not as important in the rooting of cuttings as it is commonly considered, studies regarding callus formation are likely to prove of importance, especially for such light as they may shed upon the callusing of grafts. In practical horticulture, at least two very important desiderata are here suggested: (1) The production of sufficient callus to make a mechanically and physiologically satisfactory union, and, (2) the prevention of such callus overgrowths at the union as are frequently confused with the galls brought about by the crown-gall organism, *Bacterium tumefaciens* S. and T.

Records of callus formation were not made for the intermediate temperatures of experiments 1 and 2. In experiment 3 (Table 1), with flowing air and bases of cuttings on wet cotton, there was practically no callus formation either at bases or tops, and the very slight callusing observed occurred at the tops of 3 of the 10 cuttings with maintained temperature of 30.3° C. In experiment 4, however (Table 1), which was like experiment 3 except that wire stilts were used instead of wet cotton, there was pronounced callusing at intermediate temperatures, and the graph of callus formation in this series (fig. 2, 4BT) has the form of growth-temperature curves in general. No differences between top callus and base callus were observed in this case, although such differences were shown for all the subsequent experiments with apple cuttings, as will be seen from the tabulated data. The graph indicates an optimum temperature of about 26° C. and minimum and maximum temperatures of about 18° and 40°, respectively. On comparing this callus graph with the corresponding graph for rooting (fig. 2, 4R), it is seen that the two are

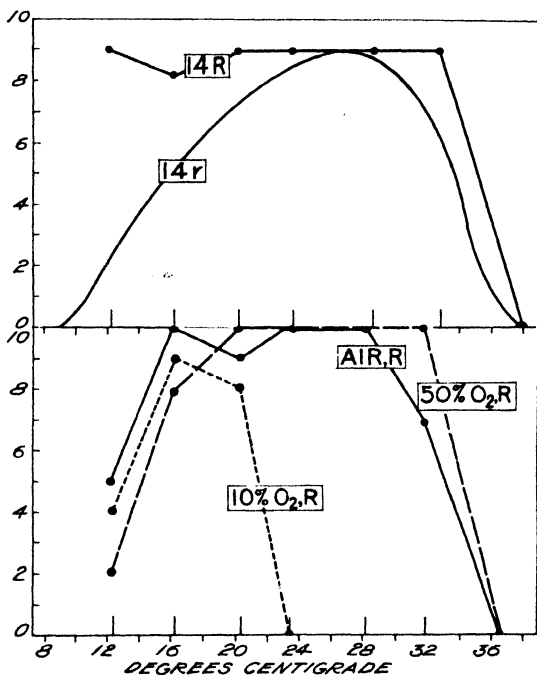


FIGURE 10.—Upper portion: Rooting of apple cuttings in closed chambers with flowing air, the cuttings having been preliminarily soaked one hour; 14R, the numerical results of experiment 14; 14r, estimated curve of actual rooting, based on experiment 14 and additional observations. Lower portion: Rooting of apple cuttings in sealed chambers filled with air, equal parts oxygen and nitrogen, or with 10 per cent oxygen in nitrogen, at the start of the test, no more interchange of gas taking place

very similar in form as well as in height, although it appears that the optimum temperature was somewhat higher for rooting than for callusing; but the rooting optimum shown for this experiment is unusually high for such optima.

That callus was plentifully formed at the intermediate temperatures in experiment 4 but was practically inhibited at all temperatures in experiment 3 suggests that this apparent inhibition may have been due to excess of water about the cutting bases in the latter case. An extra experiment was carried out with but a single temperature (27° C.), which showed that callus formation might be secured in spite of the presence of wet cotton about the bases of the cuttings. In this case there were four treatments, all with wet cotton at the bases but differing with respect to the rate of air movement through the bottles. The most rapid air flow was like that of experiment 3 (300 c. c. per hour), and the other rates were progressively lower (50, 20, and 10 c. c. per hour, respectively).

As in experiment 3, no base callus was observed with any of these four treatments, and top callus was extremely meager and practically negligible for the higher rates of air flow. However, it seems significant that the two tests with slowest air flow (20 and 10 c. c. per hour) showed top callus unquestionably more in evidence than it was in the cases where air flow was more rapid. Seven out of 10 cuttings showed small amounts of top callus in each of these cases. This extra experiment suggests that the retardation of top callus indicated by the results of experiment 3 might be offset to some extent with slower air change than was employed in experiment 3. Such a relation between callusing and rate of air flow might conceivably be due to differences in the evaporating power of the air corresponding to the different rates of flow, but this seems unlikely, since the air was practically saturated with water vapor in all these tests. It is suggested that the differences in top-callus formation here in question may have been due to differences in the oxygen supply corresponding to the differences in rate of flow. According to such a supposition, in support of which additional evidence will be given below, the extreme retardation in top-callus formation shown by the results of experiment 3 and by those of these two special tests with relatively high rates of air flow, may be regarded as due to too-rapid supply of oxygen. When air was supplied at a very slow rate (20 or 10 c. c. per hour), top callus developed much better than when the rate was more rapid (50 or 300 c. c. per hour). Other *a priori* considerations would require attention, however, if the interesting question thus raised were to be seriously studied. For an example of such considerations, products given off by the cuttings or by microorganisms might be more rapidly removed from the bottles with more rapid air renewal than from those with slower flow.

Perhaps the following statement may express the indications from experiments 3 and 4 and from the extra series: It appears that an excess of water about the bases of the cuttings inhibited callus formation at the bases and greatly retarded it at the tops, even for maintained temperatures in the optimal range, but that this retardation tended to disappear when the oxygen supply was much lower than in experiment 3.

In experiments 5 and 6 (Table 1) callusing was very active at both base and top for intermediate temperatures. It was about 33 per

cent less active at top than at base in the optimal temperature range. Graphs for the callus values from these two experiments are shown in Figure 4. In these cases the flowing gas was a mixture of oxygen (5 per cent) and nitrogen (95 per cent), and the rate of flow was more rapid (300 c. c. per hour) in experiment 5 than in experiment 6 (50 c. c. per hour). The minimum and maximum temperatures appear to have been about 13° and 38° for top callus, and the optimum with the slower flow of gas is indicated as about 28° for both base and top. In their optimal ranges the graphs for rapid gas flow are much flatter across the top than are the graphs for slow flow. Of the two rates of oxygen supply here tested, the more rapid rate produced more callus for sub-optimal and supra-optimal temperatures than did the slower rate, and it is therefore possible that a still more rapid rate of flow might have given an optimal range extending somewhat farther to the right, since the optimum rate of oxygen supply should be greater for the upper portion of the temperature range dealt with than for the lower portion. It is remarkable that a definite temperature optimum is not shown by either graph of experiment 5, although optima (about 28°) are clearly indicated by both graphs of experiment 6. From the results for 32.8°, it seems as if callusing at both top and base may be retarded by insufficient oxygen supply.

A comparison of callusing with rooting in experiments 5 and 6 indicates that the optimum and maximum temperatures were lower for rooting than for callusing. It is suggested that the minimum rate of oxygen supply necessary for rooting is significantly higher than the corresponding minimum rate for callusing.

Experiment 7 (fig. 5) with flowing oxygen gave more base callus than top callus, but callus formation was markedly retarded at all temperatures tested, as compared with the results of experiments 4, 5, and 6. From experiment 7 the highest score value for callusing is 3 (temperature about 30°) while the corresponding best callus score for experiment 4 is 5 (temperature about 26°) and for both experiments 5 and 6 it is 9 (temperature about 23° to 29°). A reason for this may be that in experiment 7 the oxygen supply was too great for excellent callusing at any temperature employed. It seems to be significant that the high oxygen supply of this test gave less callusing about the optimum temperature than was shown in any of the other experiments. It appears as though, for optimal temperatures and adequate moisture conditions, callus formation may differ from rooting by not requiring so much oxygen, and also by requiring an actually lower supply of oxygen. In other words, the lower limit of the range of oxygen supply that permits best growth may be lower for callusing than for rooting, and the extent of this range (from its lower to its upper limit) may be shorter for callusing than for rooting.

Experiments 8 and 9 in which flowing air was used were alike except for preliminary water treatment. The cuttings of experiment 8 had been injected with water and then completely submerged for 22 hours, whereas those of experiment 9 had not been injected, and only their bases had been submerged for the 22-hour period. No callusing was observed in experiment 8, but good callus formation occurred at the optimal temperatures in experiment 9 (fig. 6), which gave a maximum callus score of 6 (temperature about 29°). From these two comparative tests it appears that excessive preliminary water treatment greatly retarded rooting and completely inhibited callusing.

The graph of callusing for experiment 9 (fig. 6) is in general typical, showing a marked tendency for base callus to surpass top callus. The minimum temperature is shown as somewhat lower for base callus (about 14°) than for top callus (about 18°), the maximum temperature is the same for both base and top callus (about 39°), and the optimal region extends from about 25° to about 30° . The treatment of experiment 9 seems to have been very good for both rooting and callusing, but not so good for callusing as were the treatments of experiments 5 and 6.

Experiments 10, 11, and 12, the last two being duplicates and all three having flowing air, constitute another test of the influence of preliminary water treatment along the subsequent gas treatment of flowing air. The cuttings of experiment 10 had their bases soaked 19 hours, while those of experiments 11 and 12 had their bases first injected with water and then soaked 19 hours. The graphs of these data are shown in Figure 7. It is not clear that any significant difference was brought out. As in the case of rooting in the same experiments, both treatments appear to have been good. The usual difference between top and base callus is brought out for both of these water treatments, and the temperature optimum for callusing is shown as about 29° for both treatments.

Experiments 13, 14, 15, and 16 were planned to obtain further evidence on the effect of preliminary water treatment on callusing as well as on rooting. The data for callus formation are presented graphically in Figure 9, and may be compared with those for rooting shown in Figure 8. The four treatments, all with flowing air, were as follows: Without preliminary water treatment (experiment 13); cuttings completely submerged in water for 1 hour (experiment 14); bases soaked in water for 24 hours (experiment 15); bases soaked in water for 48 hours (experiment 16). Although the evidence is not as clear as it might have been had this series been repeated several times so that averages could be secured, yet a study of Figure 9 indicates that in general both base and top callus were markedly decreased by soaking and that the retarding effect upon callusing seemed to be at least roughly proportional to the amount of soaking.

Excessive preliminary water treatment is again shown to diminish callus formation at each temperature within the total temperature range, without shifting of the optimum temperature. For rooting (fig. 8) the treatment of experiment 14 was clearly better than that of experiment 13, the standard treatment. The same difference was noted for callusing, but in the opposite direction. It seems to be indicated that while certain preliminary water treatments were beneficial for rooting, callusing was retarded, even by comparatively moderate water treatments, and this is spite of the indications already referred to showing that low oxygen supply (quite obviously associated with high water supply) favors callusing.

The excessive preliminary water treatments tested (experiments 15 and 16) showed retardation with respect to both rooting and callusing, but this effect was much more pronounced in the case of callusing.

In these experiments, as is true in general, base callus tended to be somewhat more pronounced than top callus when both were vigorous. But experiments 15 and 16, with excessive preliminary water treatments and low values for callus throughout, showed top callus surpassing base callus for the maintained temperature about 29° C. It

seems that the extra and excessive water treatment of the bases in these two cases retarded callus formation at both top and base, but not so pronouncedly at top as at base, where the water was applied. Exactly in what way this water treatment acted to retard or inhibit callus formation can not be stated. There are some reasons for believing that the presumable excess of water supplied to the tissues did not operate merely to retard movement of oxygen. The prolonged soaking may have removed some materials from the basal tissues, or other things may have happened. Almost all the cuttings of experiment 3, which had their bases surrounded by water (wet cotton) throughout the entire experiment period, failed to produce any callus at either base or top. The base of each cutting was subjected to continuous soaking, more prolonged than in the case of experiments 15 and 16. On the whole, the retardation in callus production seemed to vary directly with the length of the soaking periods, as has been noted.

SUMMARY OF RESULTS OF EXPERIMENTS 1 TO 16

The main numerical results of callusing, especially with regard to the cardinal temperatures, are summarized in Table 2 along with the corresponding data for rooting. The minimum temperature for callusing was several degrees higher than the minimum for rooting. In the case of rooting some activity was observed at the lowest temperature in every experiment started after the middle of January, but in the case of top and base callusing the cuttings used in the 16 experiments gave no evidence of activity at the lowest temperatures.

The minimum temperature for base-callus formation was not far from 16° C.; no callus was observed on cuttings when any temperature below 16.2° was used, and the amount of callus that occurred at any temperature below 20° had in no case an index value higher than 3.

In most of the experiments the optimum for base-callus formation was 29° C. The corresponding optimum for rooting lay between 26° and 28°; hence the optimum for base callusing was slightly higher than that for rooting.

Except in experiments 3, 8, 15, and 16, in each of which the cuttings had been given some excessive water treatment, the maximum temperature for base callusing was about 38° C. The maximum temperature was about the same for base callusing and rooting. Experiments 5 and 6 are exceptions to this, however, for in these cases the maximum temperature for rooting was lowered to about 34°, but no such effect was evident in the case of callusing. These exceptions are probably due to the low oxygen supply in these two experiments. The minimum oxygen supply was higher for rooting than for callusing.

In general, base callusing was more pronounced than top callusing, and this seems in some measure to have been responsible for the narrower temperature range indicated for top callusing. When nontemperature conditions are considered as not limiting, the apparent range for top callus is about 20° to 38° C., whereas that for base callus is about 16° to 38°. Whether the upper limit of these two apparent ranges (38°) is really the upper limit of the absolute range

for each process can not be definitely stated, but it seems likely that 38° is approximately the highest temperature at which any callus formation at either end of the cutting would occur, no matter what length of period was employed.

Apparently, no top callusing took place at any temperature below 20.3° C., and the most that occurred at any temperature below 23° is represented by an index value of 4 (experiment 4); thus the minimum temperature for formation of top callus was not far from 20° in these experiments. On the other hand, the minimum for the formation of base callus was about 16°.

The absence of callus on the cuttings of experiment 3 and on those at some of the maintained temperatures of experiments 15 and 16 does not imply that base callus can not ultimately develop when the cutting bases are water-logged, for considerable base callus was observed to have formed under water in some long-time experiments not here reported.

The studies of Knight (9) indicate that callusing was actually favored by water in his tests, for he observed more callusing in wet clay soils than in drier sandy soils. Knight's observations extended over several months rather than a few days, and in these experiments also the time factor seems to have played an important rôle. The observations of Knight seem to be in agreement with those of the present writer, if the suggestions be accepted that callusing is greatly retarded, but not inhibited by excessive amounts of water, and that the most important part played by sand in retarding callusing comes from its obviously very much higher oxygen supply.

Some evidence was secured indicating a seasonal relation of callusing similar to that shown for rooting (fig. 3) although this relation was not seriously studied for either callusing or rooting. For like treatments, experiment 13 (begun February 26) gave generally higher callus values than did experiment 4 (begun December 7). It appears that both rooting and callusing are more vigorous in the spring than in the fall, other conditions being similar and favorable, and of course the same is known to be true for the pushing of buds in such forms as apple.

No effect of buds upon the rooting or callusing of cuttings was observed. It appeared that rooting was confined entirely to the primordia visible on the cutting at the time it was taken from the tree, and that most of these primordia rooted, irrespective of the presence or absence of buds on the cutting. In almost all the tests callusing appeared to be closely associated with rooting, with certain environmental differences indicated, however, for optimal rooting and optimal callusing. A slightly higher temperature, a somewhat lower water supply, and a somewhat lower oxygen supply seemed to be required for optimal callus formation than for optimal root formation.

ADDITIONAL EXPERIMENTS ON ROOTING AND CALLUSING OF APPLE CUTTINGS

Several additional experiments with apple cuttings were performed, in some of which gas did not flow continuously through the experiment chambers. In these tests, chambers of different sizes or gas mixtures with different percentages of oxygen were used, the number of cuttings being the same in the different chambers of any series. In other cases flowing air was used, or the air of the chamber was renewed from time to time. In some experiments several different maintained temper-

atures were employed, and in others the temperature was the same for all chambers. Several different water treatments were used.

Three of these additional experiments (Nos. 17, 18, and 19) were performed with temperature series similar to those used in experiments 1 to 16. For these experiments, sealed chambers of about 1,000 c. c. capacity were used. The chambers were filled with a known gas mixture when the cuttings were inserted, and there was no gas flow. For experiment 17 the gas mixture was half oxygen and half nitrogen, for experiment 18 it was ordinary air, and for experiment 19 it was 90 per cent nitrogen and 10 per cent oxygen. All cuttings were soaked for one hour before they were placed in chambers.

The numerical data from these three experiments are presented in Table 3 and are graphically shown in the lower part of Figure 10. These results seem to emphasize further the suggestion obtained from the experiments with flowing gases: namely, that the oxygen supply requisite for optimum rooting or callusing is different for different temperatures. With the lowest oxygen supply (the 10 per cent mixture) the maximum temperature was about 23° C., but for the other two gas treatments (21 per cent and 50 per cent oxygen) the maximum was about 37°. For the lowest supply of oxygen, the optimum was about 17°, and for the two other gas treatments a broad optimal range of temperatures is evident, extending as far as 32° for the 50 per cent mixture.

TABLE 3.—Numerical values for pushing of roots and growth of callus of apple cuttings in sealed chambers, without flowing gas

Experiment No.	Conditions	Temperature	Callus				Temperature	Roots	Callus				Temperature	Roots	Callus					
			Roots	Top	Base	Lenticel			Roots	Top	Base	Lenticel			Roots	Top	Base	Lenticel		
17	50 per cent O ₂	°C.	12.4	2	0	0	3	°C.	16.3	8	0	0	5	°C.	20.4	10	0	0	2	8
18	Air.....	12.4	5	0	0	0	3	16.3	10	0	0	5	20.4	9	0	0	2	3	7	
19	10 per cent O ₂	12.4	4	0	0	0	3	16.3	9	0	0	5	20.4	8	0	2	3	3	5	

Experiment No.	Conditions	Temperature	Roots	Callus				Temperature	Roots	Top	Base	Lenticel	Temperature	Roots	Top	Base	Lenticel	Temperature of highest chamber (no growth)	
				Top	Base	Lenticel													
17	50 per cent O ₂	°C.	10	0	3	9	°C.	28.3	10	0	3	9	°C.	31.9	10	0	0	9	36.7
18	Air.....	23.5	10	2	4	9	28.3	10	2	6	9	31.9	7	0	0	0	4	36.7	
19	10 per cent O ₂	23.5	0	0	0	2	28.3	0	0	0	2	31.9	0	0	0	0	2	36.7	

Four additional experiments (Nos. 20, 21, 22, and 23) involved several different water and gas treatments, all carried out in the dark room at a temperature of about 19° C. All of these experiments began February 5, 1927, and for each one 23 cuttings were used. The several treatments and the rooting score values are shown in Table 4. It seems to be clearly indicated here, as in the results of experiments 3 and 8, that any considerable amount of wetting was detrimental to root pushing.

TABLE 4.—*Effect of water treatment on pushing of apple roots at about 19° C*

[Started on February 5; each test contained 23 cuttings]

Experiment No.	Preliminary treatment	Gas treatment	Roots (scale of 10)
20	None	Flowing, saturated air	9
21	do	Moist, open air; bases occasionally wetted	8
22	Soaked 24 hours	Flowing, saturated air	1
23	None	Nearly saturated air, changed daily; cuttings immersed 10 minutes daily.	.5

Two other experiments (Nos. 24 and 25) were also conducted in the dark room at a temperature of about 19° C., beginning March 4, 1927. Sealed chambers of different sizes were used, without any change of gas during the experiment period. In experiment 24 the chambers were originally filled with air, and in experiment 25, with oxygen. Twenty-five cuttings were used for each chamber, their bases having been soaked 24 hours before the test started. Each experiment gave comparisons between five different sizes of chambers. The volumes of the chambers and the score values for root pushing and for the production of top, base, and lenticel callus, as secured at the end of the 10-day period, are given in Table 5.

TABLE 5.—*Numerical values for pushing of roots and for growth of callus in apple cuttings in sealed chambers, with different gas mixtures, at a temperature of about 19° C.*

[Started on March 4; the bases of all cuttings were soaked 24 hours]

Experiment No.	Gas used	Volume of sealed chamber	Roots	Callus		
				Top	Base	Lenticels
		C. c.				
24-A	Air	5,400	10	0	3	8
24-B	do	3,000	10	0	3	8
24-C	do	2,700	10	0	2	6
24-D	do	1,800	7	0	1	4
24-E	do	1,000	1	0	0	2
25-A	Oxygen	5,400	10	0	0	7
25-B	do	3,000	9	0	0	6
25-C	do	2,700	9	0	0	5
25-D	do	1,800	10	0	0	3
25-E	do	1,000	3	0	0	2

In addition to offering indications as to the supply of oxygen necessary for optimum root pushing, these data show (1) that more base callus than top callus was formed and (2) that large supplies of oxygen had retarded callus formation at both ends of the cutting.

In experiment 26 (started October 18) the chambers used were large-mouthed bottles. The air in one bottle was swept out by oxygen, and a rubber bag containing oxygen under slightly more than atmospheric pressure was then connected and allowed to remain throughout the test; a second bottle was arranged in the same way, but oxygen was passed through it for a short period every day; the third bottle was filled with air at the start and was left with two small openings through which some gas exchange with the external air might occur. All cuttings rested with their bases upon damp cotton, and all bottles stood on a bench in the greenhouse without intense

sunlight. After three weeks all visible primordia had pushed except those in the sealed bottle with oxygen; here only a trace of activity was observed. It seemed quite evident that lack of oxygen rather than surplus of oxygen was responsible for this marked inhibition of rooting.

In two preliminary experiments the apple cuttings in some chambers were subjected to carbon dioxide (from a commercial tank) with nearly an atmosphere of pressure, while in other chambers the cuttings were subjected to nearly pure nitrogen. Both of these treatments showed injury as indicated by tissue disintegration in the bark and wood, but the cuttings treated with carbon dioxide were much more seriously injured than the others. In both cases injury was especially pronounced and rapid in the series of cuttings which bore leaves. Here, as in those without leaves, injury was evident sooner with carbon dioxide than with nitrogen.

The results of these additional experiments seemed to support the general conclusions reached in the main series, namely, that apple cuttings have a higher oxygen requirement for rooting than for callusing, and that any study of the oxygen supply required for rooting or callusing must include special consideration of the temperature. The experiments without flowing gas are to be considered as only superficial in their indications, because of the fact that the nature of the gas mixture undoubtedly changed during the experiment period and in an unknown manner. The oxygen content must have been decreased more or less rapidly through the respiration of the cuttings themselves and perhaps to a considerable degree by the oxidation occurring on the iron wire used as supports.

EXPERIMENTS WITH WILLOW CUTTINGS

The studies on willow cuttings were about equally divided between experiments with flowing gases and experiments with sealed chambers. The experiments with flowing gases will be considered first. Willow cuttings were present in the chambers of the first 12 experiments with apple cuttings.

The numerical results of these 12 experiments on willow are presented in Table 6. In addition to the numerical scores for rooting, derived as has been described for the experiments with apple cuttings, the results for willow include another value representing rooting activity, namely, the average number of roots per cutting evident at the end of the experiment. These additional values are given in the table in parentheses following the regular score values. Thus for experiment 5, for temperatures 20.6°, 23.6°, 28.6°, and 32.8° C., the root scores are, respectively, 1, 4, 5, and 5, and the new root values (in parentheses) are 0.3, 1.1, 1.7, and 1.0. In the third case, for instance, the score 5 indicates that half of the cuttings showed some root pushing, and the value 1.7 is the quotient obtained by dividing the total number of roots observed by the number of cuttings used.

Table 7 gives the numerical results of an experiment run at the same time as experiment 3. Here, however, only one temperature was used, and different rates of air supply were compared. These results will be considered only as regards callus formation, for rooting was similar in this case to that in experiment 3.

TABLE 6.—Numerical values for pushing of roots and for growth of callus of willow cuttings

Experiment No.	Date started	Conditions	Pushing of roots •				Roots •				Callus ^a				Roots •				Callus ^b				Roots •				Callus ^a				Temperature of high-growth chamber, no				
			Temp	Top	Base	Lenticel	Temp	Roots •	Temp	Top	Base	Lenticel	Temp	Roots •	Temp	Top	Base	Lenticel	Temp	Roots •	Temp	Top	Base	Lenticel	Temp	Roots •	Temp	Top	Base	Lenticel					
1	1926 Nov. 8	Flowing O ₂ ; bases on damp cotton	7.7	0	0	0	14.7	0	0	0	220.7	4	(-)	827.7	9	(+)	834.1	5	(+)	(+)	339.8	0	0	0	0	0	0	0	0	0	0	46.5			
2	Nov. 23	Flowing air; bases on damp cotton	13.2	0	0	0	17.2	0	0	121.9	5	0	1	425.7	7	1	2	531.3	1	8	9	036.0	1	0	0	5	40.7	1	0	0	5	40.7			
3	Dec. 7	Flowing air; bases on wet cotton	14.1	0	0	0	217.8	0	0	422.1	10	0	0	625.5	10	0	0	830.3	10	1	0	835.3	10	0	0	6	40.2	10	0	0	6	40.2			
4	do.	Flowing air; bases on wire stills	14.1	0	0	0	117.8	0	1	0	422.1	6	5	5	625.5	10	5	5	630.3	8	5	3	535.3	1	2	3	2	40.2	1	2	3	2	40.2		
5	Jan. 5	Flowing 5 per cent O ₂ ; fast stills	13.3	0	0	0	116.7	0	0	120.6	1	(0.3)	0	223.6	4	(1.1)	3	5	328.6	5	(1.7)	6	432.8	5	(1.0)	5	5	4	37.9	5	(1.0)	5	5	4	37.9
6	do.	Flowing 5 per cent O ₂ ; slow stills	13.3	0	0	0	116.7	0	0	220.6	2	(0.7)	0	2	623.6	7	(2.5)	5	5	828.6	5	(2.1)	5	5	832.8	9	(3.0)	5	5	8	37.9	37.9			
7	Jan. 21	Flowing O ₂ ; stills	14.4	0	0	0	117.9	0	0	221.7	0	(0.3)	1	2	524.8	3	(0.8)	2	3	329.7	3	(1.2)	1	0	333.8	1	(0.2)	0	1	38.9	1	(0.2)	0	1	38.9
8	Feb. 3	Flowing air; stills; injected 22 hours	14.2	0	0	0	517.7	8	(3.0)	0	621.4	10	(0.0)	0	5	724.5	10	(15.7)	3	5	829.6	10	(18.4)	2	5	833.7	10	(9.5)	0	3	7	39.0	39.0		
9	do.	Flowing air; stills; bases soaked 22 hours	14.2	0	0	0	417.7	6	(1.5)	2	521.4	9	(5.2)	0	4	724.5	10	(12.1)	4	7	729.6	10	(12.3)	5	8	733.7	10	(13.7)	0	2	7	39.0	39.0		
10	Feb. 15	Flowing air; stills; injected 15 hours	12.4	0	0	0	316.2	6	(1.7)	0	520.3	10	(14.7)	0	0	(-)	23.4	10	(14.7)	0	(-)	23.4	10	(21.2)	0	(-)	32.8	10	(18.0)	0	5	(-)	37.6	37.6	
11	do.	Flowing air; dry 22 hours; injected 25 minutes	12.4	0	0	0	316.2	7	(2.3)	0	520.3	10	(12.8)	0	0	(-)	23.4	9	(17.3)	0	(-)	23.4	10	(19.7)	0	(-)	32.8	10	(20.0)	0	5	(-)	37.6	37.6	
12	do.	Flowing air; dry 22 hours; injected 25 minutes	12.4	0	0	0	316.2	6	(2.6)	0	520.3	10	(16.3)	0	2	(-)	23.4	9	(9.6)	0	(-)	23.4	10	(19.5)	0	(-)	32.8	10	(10.3)	0	5	(-)	37.6	37.6	
		Average of 11 and 12	12.4	0	0	0	316.2	6.5	(2.5)	0	520.3	10	(14.6)	0	1	(-)	23.4	9	(13.5)	0	(-)	23.4	10	(19.6)	0	(-)	32.8	10	(15.2)	0	5	(-)	37.6	37.6	

* The figures in this column represent the proportion of cuttings that showed some pushing of roots at the end of 10 days, calculated on the basis of 10. The figures in parentheses show the average number of roots per cutting evident at the end of the 10-day period.

* The figures in these columns represent the total amount of callus shown by all the cuttings in each lot (not the number of cuttings that showed some callus), based on an arbitrary scale of 10.

* No observation.

TABLE 7.—Numerical values for pushing of roots and growth of callus of willow cuttings with a maintained temperature of 27° C. and with different rates of flowing gas

[Started on December 7, 1926; all cuttings with wet cotton around bases]

Experiment	Speed of air flow	Roots	Callus		
			Top	Base	Lenticel
A	300 c. c. per hour.....	10	0	0	7
B	50 c. c. per hour.....	10	2	0	7
C	20 c. c. per hour.....	10	6	0	7
D	10 c. c. per hour.....	9	6	1	7

Table 8 shows the data from all experimentation with the seven different maintained temperatures when flowing gases were not used; each chamber was filled with the gas indicated and then sealed. Experiment 7-A is so numbered because it was run at the same time as experiment 7. Experiments 13 to 16 were all started February 26, 1927.

TABLE 8.—Numerical values for pushing of roots and for growth of callus of willow cuttings with maintained temperatures, without flowing gases

Experiment No.	Gas at start	Date started	Treatment	Temperature	Callus			Temperature	Callus			Temperature	Callus		
					Roots	Top	Base		Roots	Top	Base		Roots	Top	Base
7-A	Oxygen	1927 Jan. 21	Stilts	° C.				° C.				° C.			
13	Air	Feb. 26	do	14.4	0	0	0	17.9	1	0	0	21.7	4	0	0
14	do	do	do	12.2	0	5	5	16.2	1	8	8	20.3	2	10	10
15	do	do	do	12.2	0	0	0	16.2	1	6	0	20.3	1	9	0
16	do	do	do	12.2	0	0	0	16.2	1	0	0	20.3	1	3	3
	do	do	do	12.2	0	0	0	16.2	1	2	2	20.3	4	3	3

Experiment No.	Gas at start	Date started	Treatment	Temperature	Callus			Temperature	Callus			Temperature	Callus			Temperature of highest chamber; no growth
					Roots	Top	Base		Roots	Top	Base		Roots	Top	Base	
7-A	Oxygen	1927 Jan. 21	Stilts	° C.				° C.				° C.				° C.
13	Air	Feb. 26	do	24.8	3	1	3	29.7	5	2	2	33.8	0	0	0	38.9
14	do	do	do	23.7	3	10	10	28.7	2	8	9	32.8	1	5	5	38.0
15	do	do	do	23.7	3	10	2	28.7	2	8	0	32.8	1	5	0	38.0
16	do	do	do	23.7	3	3	4	28.7	1	3	4	32.8	0	0	0	38.0
	do	do	do	23.7	4	3	4	28.7	1	6	4	32.8	1	3	4	38.0

Table 9 presents the numerical results of all experimentation with willow in which the single-chamber constant-temperature apparatus was used except those given in Table 7. In none of the experiments referred to in Table 9 was flowing gas used; sealed chambers were employed in all cases, though in 4 of the 39 tests gas was passed through the chamber twice daily. In each of these tests the experiment period was seven days.

TABLE 9.—Numerical values for pushing of roots, for average number of roots per cutting, and for growth of callus of willow cuttings, with maintained temperatures and with different gas supplies

[All cuttings were on wire stilts, 50 cuttings in each lot]

Ex- peri- ment No.	Date started	Tem- per- ature	Prelim- inary treat- ment	Size of bottle	Gas treatment	Roots		Callus		
						Cut- tings rooted	Average roots per cut- ting	Top	Base	Lenticel
	1927	° C.		C. c.						
17-A	Jan. 8	27	None	4,200	Air, closed	6	2.5	2	3	3
17-B	do.	27	do.	4,200	Air, changed twice daily	2	.7	5	6	4
17-C	do.	27	do.	4,200	O ₂ , closed	5	1.2	1	2	3
17-D	do.	27	do.	4,200	O ₂ , changed twice daily	3	.8	0	0	2
18-A	Jan. 15	27	do.	4,200	5 per cent O ₂ , closed	0	.3	0	0	4
18-B	do.	27	do.	4,200	5 per cent O ₂ , changed twice daily	2	.7	3	5	5
18-C	do.	27	do.	4,200	N ₂ , closed	0	.1	0	0	1
18-D	do.	27	do.	4,200	N ₂ , changed twice daily	0	.2	0	0	2
19-A	Jan. 22	27	do.	4,200	Air, closed	8	5.5	5	6	7
19-B	do.	27	do.	4,200	29 per cent O ₂ , closed	7	3.7	5	6	7
19-C	do.	27	do.	4,200	38 per cent O ₂ , closed	8	3.7	5	6	7
19-D	do.	27	do.	4,200	12 per cent O ₂ , closed	1	.5	3	4	7
20-A	Jan. 29	26.5	do.	4,200	Air, closed	8	3.7	5	6	7
20-B	do.	26.5	do.	4,200	21 per cent O ₂ , closed	9	3.3	5	6	7
20-C	do.	26.5	do.	4,200	17.5 per cent O ₂ , closed	3	1.0	4	5	7
20-D	do.	26.5	do.	4,200	14 per cent O ₂ , closed	1	.5	3	4	7
21-A	Feb. 5	29	do.	4,200	Air, closed	7	4.9	5	6	7
21-B	do.	29	do.	4,200	50 per cent O ₂ , closed	7	4.4	5	6	7
21-C	do.	29	do.	4,200	75 per cent O ₂ , closed	8	6.9	4	5	7
22-A	Feb. 12	29	do.	5,400	Air, closed	6	3.7	7	8	7
22-B	do.	29	do.	4,200	do.	5	3.0	7	8	7
22-C	do.	29	do.	2,700	do.	2	.5	6	7	7
22-D	do.	29	do.	2,200	do.	3	.9	7	8	7
23-A	Feb. 19	29	Injected	5,400	do.	10	11.6	0	3	7
23-B	do.	29	do.	4,200	do.	10	7.4	0	2	7
23-C	do.	29	do.	2,700	do.	1	.7	0	1	7
23-D	do.	29	do.	2,200	do.	0	.5	0	0	7
24-A	Feb. 27	29	Dry two days	4,200	do.	6	3.0	5	6	7
24-B	do.	29	do.	4,200	50 per cent O ₂ , closed	8	6.6	5	7	7
24-C	do.	29	do.	4,200	75 per cent O ₂ , closed	8	7.8	5	7	7
24-D	do.	29	do.	4,200	O ₂ , closed	9	7.1	5	7	7
25-A	Mar. 6	32	None	4,200	Air, closed	5	2.9	9	10	7
25-B	do.	32	do.	4,200	50 per cent O ₂ , closed	8	6.7	9	10	7
25-C	do.	32	do.	4,200	75 per cent O ₂ , closed	8	6.0	9	10	7
25-D	do.	32	do.	4,200	O ₂ , closed	9	7.0	9	10	7
26-A	Mar. 13	34.5	do.	4,200	Air, closed	1	.6	7	8	7
26-B	do.	34.5	do.	4,200	50 per cent O ₂ , closed	4	1.9	7	8	7
26-C	do.	34.5	do.	4,200	75 per cent O ₂ , closed	4	2.7	5	6	7
26-D	do.	34.5	do.	4,200	O ₂ , closed	4	2.5	5	6	7

As in the case of the apple data, the results with willow, especially those with maintained temperatures and flowing gases, will be discussed mainly with reference to the corresponding graphs, Figures 11 to 17. In those graphs (figs. 15, 16, and 17) in which broken lines are used to represent average number of roots per cutting, the number representing the greatest average amount of root growth observed in any of these 12 experiments (21.2) has been taken as equal to 10 on the scale.

ROOTING OF WILLOW CUTTINGS

In experiment 1 (fig. 11, 1R), in which flowing oxygen was used, the total temperature range employed was much wider than the range of rooting, so that of the experiments at the seven temperatures only three showed any growth. The graph shows an optimum at 27.7° C.,

but it may have been several degrees higher or lower. The maximum appears to have been about 39° or below, while the minimum temperature is indicated as above 15° , probably below 18° .

In experiment 2 (fig. 11, 2R) the conditions of the test were similar to those of experiment 1 in that the bases of the cuttings rested on damp cotton, but in this case flowing air was used. The graph for experiment 2 shows an optimum at about 26° C., but from the data the optimum might just as well have been 28° . The maximum was about 40° or below. The minimum was slightly higher than that shown by experiment 1, being above 17° or possibly above 19° .

In experiment 3 (fig. 12, 3R) flowing air was used, and the bases of the cuttings, instead of resting on moist cotton, were surrounded to a height of about 5 cm. by wet cotton. This additional water seems to have produced a beneficial effect upon rooting, so that although maximum and minimum temperatures are indicated as before, no definite optimum temperature is apparent; the entire graph between the maximum and the minimum is thus raised to the top, for every cutting in each of the four chambers between 22.1° and 35.3° C.

showed pushing of roots. However, notes on this experiment show that among the four temperatures more roots per cutting as well as longer roots were present at 30.3° than at 25.5° . With respect to these criteria, 22° seemed to be about equal to 35° , but with both the latter temperatures less rooting occurred than with 25.5° . Therefore, it seems that the additional water treatment of experiment 3 greatly increased rooting within the total temperature range, but did not appreciably alter the previously observed maximum, optimum, or minimum, here seen to be about 39° , 29° , and 18° , respectively.

Experiment 4 (fig. 12, 4R) was a duplicate of experiment 3, except that in experiment 4 the cuttings were supported on wire stilts so that no water came in contact with the cuttings. Again there is a temperature curve similar to the other curves discussed, with maximum and minimum not far from 40° and 18° C., respectively, and with an optimum indicated at 25.5° , but which may well have been

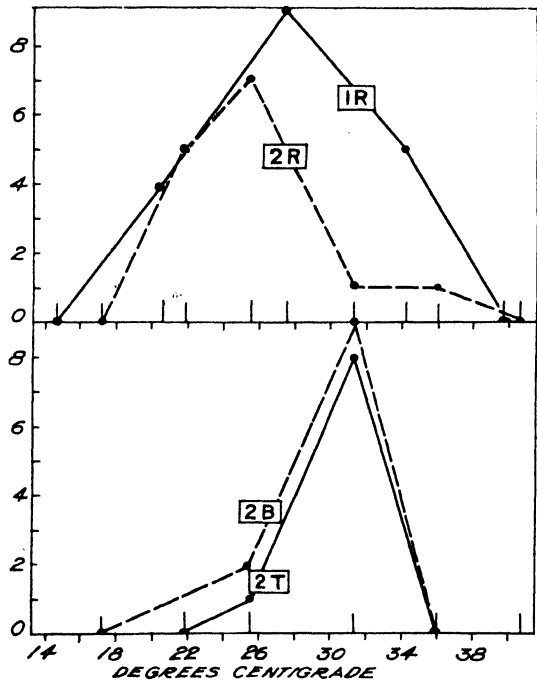


FIGURE 11. —Rooting (1R, 2R), top-callus formation (2T), and base-callus formation (2B) of willow cuttings in closed chambers with flowing oxygen (experiment 1) or with flowing air (experiment 2)

about 29°. While all cuttings did not root at 30.3°, this temperature was here, as in experiment 3, a slightly better one for the subsequent growth of the roots that did push than was 25.5°. Although the curve is for some unknown reason higher in experiment 4 than in experiment 2, still rooting in experiment 4 was much less than that observed in experiment 3, where the cuttings quite certainly were furnished with some water from the cotton. Apparently the beneficial effects of the water treatment were greatest at the supra-optimal temperatures rather than at the sub-optimal temperatures, as was observed in the corresponding experiment for apple rooting.

In experiments 5 and 6 (fig. 13) all cuttings were on wire stilts, and a flowing gas mixture containing 5 per cent oxygen was used. A

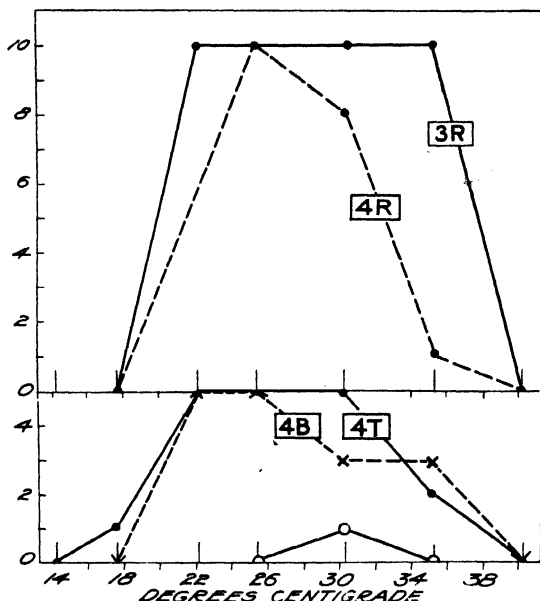


FIGURE 12.—Rooting (3R, 4R), top-callus formation (4T), and base-callus formation (4B) of willow cuttings in closed chambers with flowing air: Experiment 3, with the bases of the cuttings on wet cotton; experiment 4, with the bases on wire stilts. With the wetter conditions around the cuttings of experiment 3, only a trace of callus appeared

rate of flow of about 300 c. c. per hour was employed in experiment 6, while a rate of about 50 c. c. per hour was used in experiment 5. In marked disagreement with the corresponding curve for apple rooting (fig. 3), the very low oxygen supply in experiment 6 at 33° C. appeared ample for good rooting in willow. In fact, except for the depression at 28°, the graph for experiment 6 is not unlike the other graphs for willow cuttings that were subjected to flowing gases without having been given some water treatment.

Another interesting point shown by Figure 13 is the fact that the graph for experiment 5 lies wholly below that

for experiment 6. Supposing that the lots of cuttings for the different tests were similar, the most obvious differences between the conditions for experiments 5 and 6 are differences in amounts of water vapor and oxygen in the corresponding chambers, due to different rates of gas flow. It seems unlikely that there was any difference in the humidity of the gas about the cuttings in the two experiments. There was surely a much larger supply of oxygen in experiment 5 than in experiment 6, and it is possible that excess of oxygen may have slightly retarded rooting in the former experiments. There are, however, several possible objections to this suggestion; for example, the results of experiment 1 (with flowing oxygen) and of experiment 4 (with flowing air) appear to be in disagreement with it. It is possible that the presence of the wet cotton in experiment 1 may, in some

way, have counteracted unfavorable effects of high oxygen concentrations. This possibly injurious effect of large supplies of oxygen is also suggested in experiment 7 and in some of the experiments listed in Tables 8 and 9, in which continuously flowing gases were not used. On the basis of the supposition that large supplies of oxygen produce an injurious effect upon rooting of willow, it is hard to reconcile the results of experiment 4, where large quantities of air were passed through the chambers, where no liquid water was supplied the cuttings, and where, nevertheless, high percentages of rooting were secured.

In contrast to the corresponding results with apple, the willow cuttings in experiment 6 gave no evidence of oxygen deficiency at supra-optimal temperatures; the small amount of oxygen here supplied seemed to have been ample for good root growth, even at 32.8° C. Except with regard to the somewhat questionable optima, the graphs for experiments 5 and 6 agree with the other graphs for willow, showing maxima near 38° and minima at about 17°.

The results of experiment 7, as just mentioned, suggest that a large supply of oxygen may be detrimental to the rooting of willow cuttings. Although the usual maximum of about 38° C. is here indicated, experiment 7 (fig. 14, 7R) shows the only graph obtained in all these 12 experiments in which the minimum is above 18°; here the minimum is about 22°. The optimum (about 25°) is likewise unusual. All score values in this experiment are low, which seems to support the suggestion that an excess of oxygen may retard rooting in willow.

The striking differences brought out in Figure 14, between the results of experiment 1 (flowing oxygen, started November 8) and those of experiment 7 (flowing oxygen, started January 21) hardly seem to have been the result of seasonal differences in the capacity of the cuttings to produce roots. They seem rather to have been related either to unlike variability in the two lots of cuttings or to differences in water supply; the cuttings in experiment 1 may have

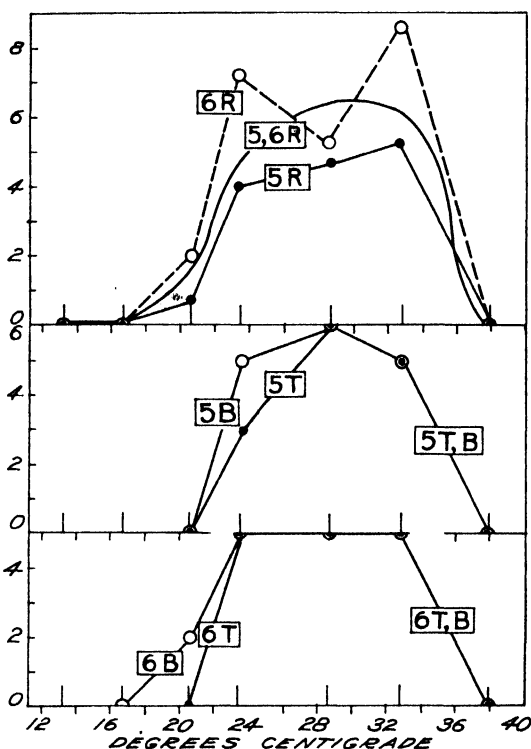


FIGURE 13.—Rooting (5R, 6R), top-callus formation (5T, 6T), and base-callus formation (5B, 6B) of willow cuttings in closed chambers with flowing 5 per cent oxygen in nitrogen

been supplied with sufficient water to counteract any injurious effect caused by surplus of oxygen.

In experiments 8 and 9 (fig. 15) the maxima and minima were similar to those shown by most of the other willow experiments (about 39° and 15° C., respectively). But in these two experiments, as in experiment 3, where also the cuttings had been treated with water, the optima are not clear, for all score values for temperatures below the maximum are 10 or only slightly below. Nevertheless, by considering the average number of roots per cutting the optima are seen to lie in the vicinity of 28°. (The broken lines—marked 8r and 9r in

fig. 15, 10r in fig. 16, and 11, 12r in fig. 17—indicate the total average number of roots per cutting, the greatest number obtained in any of these experiments—21.2 per cutting in experiment 10—being taken as 10 on the scale of ordinates used.) In contrast to results obtained with apple cuttings, willow cuttings completely soaked for 22 hours after a 15-minute injection gave decidedly higher rooting values than cuttings that had the bases only soaked for 22 hours; the latter treatment, however, gave much higher values than no water treatment at all. In fact, these

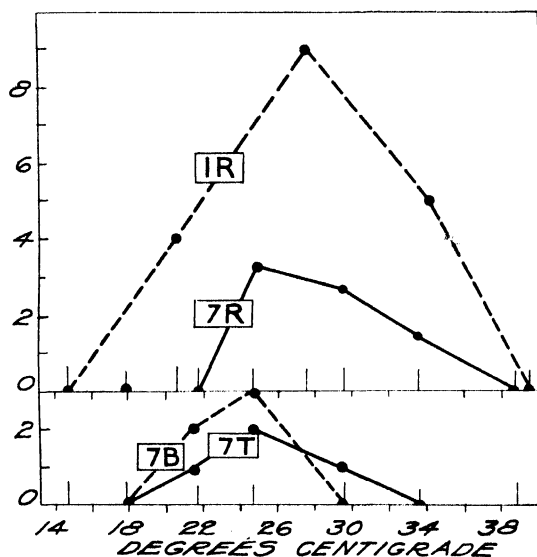


FIGURE 14.—Rooting (IR, 7R), top-callus formation (7T), and base-callus formation (7B) of willow cuttings in closed chambers with flowing oxygen. Experiment 1, bases on damp cotton; experiment 7, bases on wire stilts

willow cuttings were benefited directly in proportion to the intensity and length of the water treatment used. The only reason for supposing that prolonged water treatments at high temperatures may have been injurious (as, for example, because of lack of oxygen) arises from the fact that a slightly greater number of roots per cutting were shown by experiment 9, as compared with the number in experiment 8, at the highest temperature at which rooting occurred (33.7°). Although this difference in number of roots per cutting (13.7–9.5) is not large, it is in the direction that might be expected from the results with apple; for it seems reasonable to suppose that rooting of willow may be retarded by lack of oxygen, even though the critical limit lies far below that for rooting of apple.

The cuttings in experiments 10, 11, and 12 showed more rooting than those in the experiments with willow. Of these cuttings, those in experiment 10 (fig. 16) were given the longest water treatment and showed the greatest root growth. In experiments 11 and 12 (fig. 17) the cuttings were kept dry for one day and then given short, intensive

water treatment before being placed in the chambers; thus, whatever the total amount of water supplied to the cuttings by the two different treatments, at the end of the test the cuttings of experiment 10 had really been under rooting conditions for 10 days, as opposed to only 9 days for the others. Those used in experiments 11 and 12 received essentially identical treatments throughout, being run as duplicates in different chambers at the same temperatures.

With respect to maximum and optimum temperatures (about 37° and 29° C., respectively), the results of these three experiments (Nos. 10, 11, and 12) agree closely with those of most of the other willow tests. But these three experiments show lower minimum temperatures than any of the others with willow. A high-score value for rooting was obtained at 16.2° , and the minimum temperature for discernible root growth in 10 days lies not far from 13° .

Next to these low minima obtained in experiments 10, 11, and 12, started February 15, the lowest temperatures at which any rooting occurred were in experiments 8 and 9, started February 3; these experiments showed high-rooting values for 17.7° C. It is interesting to compare these results at a temperature of 17.7° in the two experiments last mentioned with the results at a similar temperature in experiment 3 (started December 7). The high-score values shown in both cases for other temperatures indicate that the water treatments used were satisfactory for good growth; but at a temperature of 17.8° in December no rooting whatever was evident, while at a temperature of 17.7° in February a large proportion of the cuttings rooted. As with the apple cuttings, it seems that some seasonal factor was involved which is evident in the graphs only in the vicinity of the minimum.

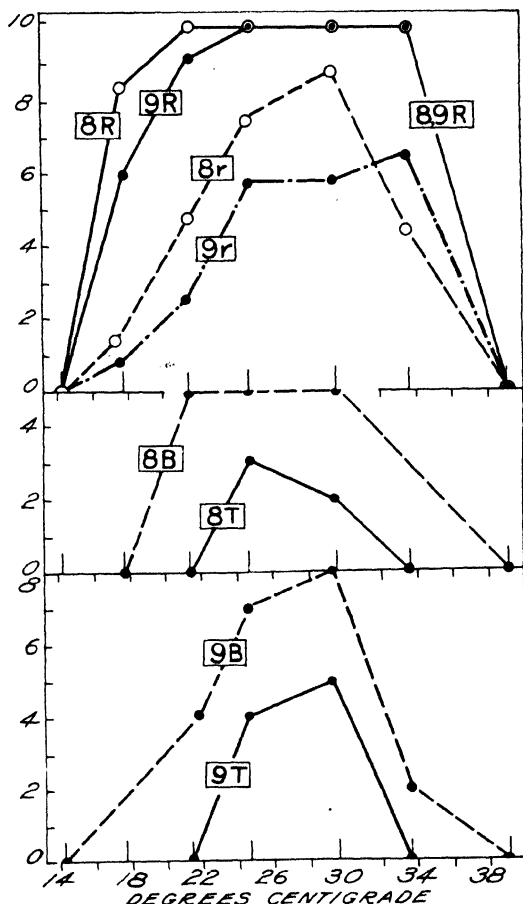


FIGURE 15.—Rooting (8R, 9R, 8r, 9r), top-callus formation (8T, 9T), and base-callus formation (8B, 9B) of willow cuttings in closed chamber with flowing air. Experiment 8, cuttings injected with water and completely immersed for 22 hours; experiment 9, bases of cuttings soaked for 22 hours

TABLE 10.—Summary of results of experiments 1 to 12 in rooting and callusing of willow cuttings

Ex- peri- ment	Date started	Flowing gas	Water conditions	Data for rooting			Data for top callusing				Data for base callusing				Data for lenticel callusing				
				Approximate car- dinal tempera- tures			Index for opti- mum tem- pera- ture	Approximate car- dinal tempera- tures			Index for opti- mum tem- pera- ture	Approximate car- dinal tempera- tures			Index for opti- mum tem- pera- ture	Approximate car- dinal tempera- tures			Index for opti- mum tem- pera- ture
				Minimum	Optimum	Maximum		Minimum	Optimum	Maximum		Minimum	Optimum	Maximum		Minimum	Optimum	Maximum	
1	1926 Nov. 8	Water saturated O ₂	Bases on damp cotton	° C. 17	° C. 27	° C. 40	9	° C. (*)	° C. (*)	° C. (*)	(*)	° C. (*)	° C. (*)	° C. (*)	(*)	° C. 10(?)	° C. 21-28	° C. 40	8
7	1927 Jan. 21	do	Cuttings not in contact with water.	22	25	38	3	18	25	34	2	18	25	29	3	10(?)	22-30	38	3
5	Jan. 5	Water-saturated mix- ture, 5 per cent O ₂ , 99 per cent N ₂	do	17	33	38	5	21	29	38	6	21	29	38	6	10(?)	29-33	38	4
6	do	do	do	17	33	38	9	21	23-33	38	5	17	23-33	38	5	10(?)	24-33	38	8
4	1926 Dec. 7	Water-saturated air	do	18	25	40	10	13	22-30	40	5	18	22-26	40	5	10(?)	22-25	40	6
2	Nov. 23	do	Bases on damp cotton	17	25	40	7	22	31	36	8	17	31	36	9	15	26	40	5
10	1927 Feb. 15	do	Injected, completely soaked 15 hours.	13	20-33	38	10	(*)	(*)	(*)		20	29	38	6	(*)	(*)	(*)	
9	Feb. 3	do	Bases soaked 22 hours	14	25-34	39	10	21	29	34	5	14	29	39	8	8(?)	22-34	39	7
11, 12	Feb. 15	do	Dry 22 hours, injected 25 minutes.	13	20-30	38	10	(*)	(*)	(*)		16	29	38	6	(*)	(*)	(*)	
3	1926 Dec. 7	do	Bases surrounded by wet cotton.	18	22-35	40	10	26	30	35	1	(*)	(*)	(*)		10(?)	25-30	40	8
8	1927 Feb. 3	do	Injected, completely soaked 22 hours.	14	21-34	39	10	21	25	3	35	18	21-29	39	5	8(?)	24-30	39	8

* No observation.

* No callus.

SUMMARY OF RESULTS OF EXPERIMENTS 1 TO 12

The main numerical results of the experiments with willow cuttings in which flowing gases and maintained temperatures were used have been brought together in Table 10, as was done for the corresponding results with apple cuttings in Table 2.

The minimum temperature for rooting of willow apparently changed from about 18°C . early in the season to about 13° later in the season. On the other hand, the maximum temperature seems to have been at all times, whatever treatment was employed, about 38° , that is, about the same as that indicated for rooting and callusing of the apple. Except for the suggestion offered in experiments 8 and 9, apparently in none of these tests with willow did the supply of oxygen fall below the critical limit, even at the higher temperatures. Hence, from these particular experiments little is seen to indicate that the supply of oxygen necessary for rooting was in any way dependent upon the temperature. However, from the results obtained without flowing gases, which will be considered later, it appears that the failure of experiments 1 to 12 to show any definite effect of oxygen deficiency may be explained by supposing that the oxygen requirement for willow is very low and was satisfactorily met in all cases where flowing gases were used.

On the basis of the rooting scores the optimum temperature is not indicated so clearly as are the maximum and minimum, for at all temperatures between 20° and 33°C . rooting was very good; judged by the average number of roots per cutting, however, the optimum temperature was about 29° .

It should be emphasized that the minimum temperature for visible root growth in 10 days was several degrees higher for willow than for apple. Although much activity was observed in apple cuttings subjected to the lowest temperatures, in every series of willow cuttings the lowest temperature employed was too low for rooting.

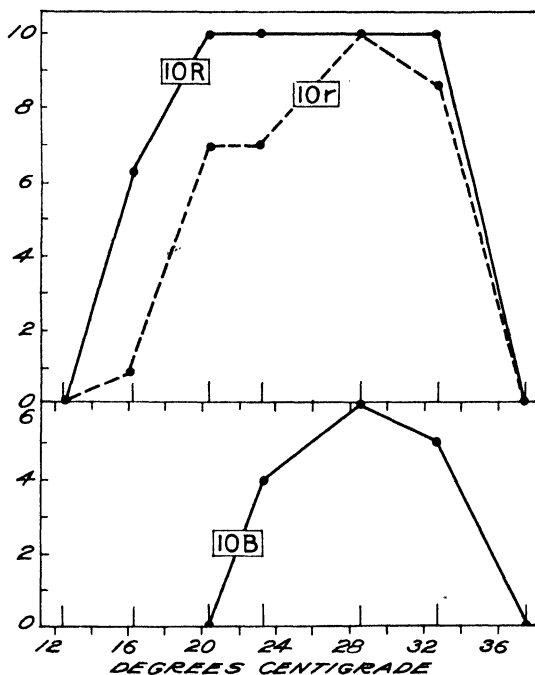


FIGURE 16.- Rooting (10R, 10r) and base-callus formation (10B) of willow cuttings in closed chambers with flowing air and with the cuttings injected with water and soaked for 15 hours at the start of the test. No top callus formed with this intensive water treatment

On the other hand, apple and willow were approximately alike in respect to their optimum and maximum temperatures. Although in these 12 experiments with flowing gases there was no indication of oxygen deficiency, the results of experiment 7 (the only experiment with flowing oxygen in which water was not available to the cuttings) suggested that the use of oxygen with a pressure of about 1 atmosphere (an oxygen excess) may have been instrumental in raising the

minimum temperature as well as in greatly decreasing the index values for all temperatures at which rooting occurred.

In regard to water supply, the results indicate that the more intense or prolonged the water treatment the greater was the rooting response. In this respect willow cuttings were very different from apple; some water treatments were highly beneficial for the rooting of apple cuttings, but more prolonged treatments were injurious.

In the experiments with willow no evidence was secured in support of the supposition that the presence of buds exerts any direct influence, as through hormones, upon root pushing.

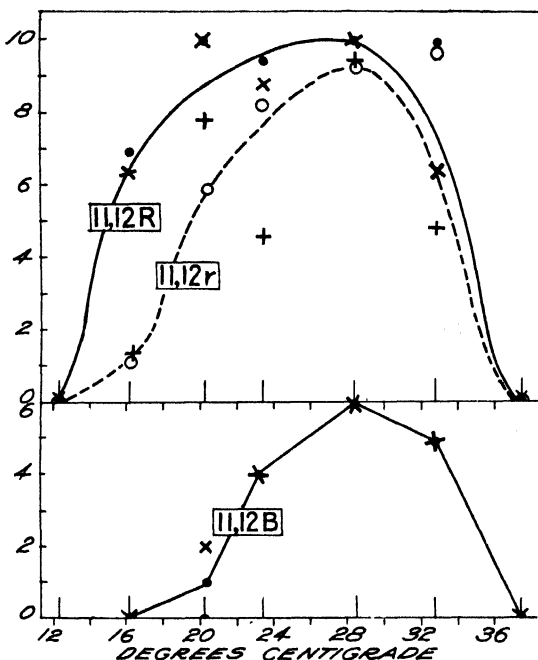


FIGURE 17.—Rooting (I1, I2R; I1, I2r) and base-callus formation (I1, I2B) of willow cuttings in closed chambers with flowing air, the cuttings having been injected with water after 22 hours of drying. No top callus formed with this treatment

Inasmuch as all of the willow cuttings bore buds, these experiments differed from those with apple cuttings in that they did not furnish any evidence against the supposition just mentioned.

CALLUSING OF WILLOW CUTTINGS

The numerical data for callusing in willow cuttings in the experiments with flowing gases are shown by graphs based on Table 6.

Except for temperature influence, no important differences in callus formation are evident in the series of gas and water treatments employed in these 12 experiments. A graph for base-callus formation with a minimum of about 18° C., an optimum of about 29°, and a maximum of about 38° answers well for all these tests except experiments 3 and 7.

In experiment 3 the only callus observed on either end was a trace of top callus produced at a temperature of 30.3° C. In itself, this almost total absence of callus on cuttings that showed a very large amount of rooting suggests again that roots and callus may reciprocate, the

activity being variously divided between rooting and callusing. However, a closer examination of the results with willow cuttings seems to indicate here, as with apple, that such reciprocation was not involved, but rather that there are actual differences between the requirements for rooting and for callusing. The bases of the cuttings in experiment 3 (being surrounded by wet cotton) were evidently too wet for callus formation, although such conditions were very good for root formation. The lack of top-callus formation in experiment 3 may, perhaps, have been due to excessive water treatment or to this water treatment in conjunction with the large oxygen supply. In the case of the special modification of experiment 3 in which different rates of air flow were employed at a single temperature (see Table 8), top callus was evident only with the lower rates of flow.

The score values for base callusing shown by experiment 7 are lower than the corresponding ones for any of the other experiments with flowing gases except experiment 3 and its modification. The extremely low maximum and optimum temperatures indicated by experiment 7, about 29° and 25° C., respectively, may have special significance, or they may show merely that all values were too low to give satisfactory temperature indications. These extremely low values for base callusing can not be explained either by a reciprocation of callusing with rooting or by excessive treatment with water. It seems that a surplus of oxygen was in some way responsible for them.

It may or it may not be significant that the only values higher than 6 for base callus were obtained in experiments 2 and 9 with cuttings that had been treated with water. While it may be that some treatment was beneficial to callusing of willow, it seems that even in willow cuttings any water treatment tended to retard callusing. This was especially evident in experiment 3 and in experiments 10, 11, and 12. In the last-mentioned experiments, although the index values for base callusing are about like those for the other experiments, top callusing was entirely inhibited by the injection treatment. In experiment 8, the other experiment in which the cuttings were injected under reduced pressure, only slight top callusing was evident; and in experiment 9, in which the cuttings were not injected but were soaked 22 hours, values for top-callus formation were more different from those for base callus than were the values shown by cuttings neither soaked nor injected. Thus in general, where the cuttings were given water treatment, the temperature range for formation of top callus lay well within the range for base callus, and in these cases the curves of minimum and maximum temperatures are not far from 21° and 34° C., respectively; also, the optima are uncertain, and all index values are low.

Except for the high value shown near 31° C. in experiment 2 and another near 29° in experiment 9, the conditions of these 12 experiments were not good for either base-callus or top-callus formation, whatever gas or water treatment was employed.

LENTICEL CALLUS

Other workers have made many studies of the external conditions influencing tissue hypertrophy around lenticels (lenticel callus). These studies have clearly demonstrated the close relationship existing between oxygen deficiency and water surplus in bringing about

lenticel hypertrophy when the temperatures are adequate, but apparently no special study has been carried out with reference to the part played by these different factors.

Although the system of score values for lenticel-callus used in the present studies is not wholly satisfactory, some indications with regard to lenticel-callus formation may be added here.

Regarding the temperature curve for lenticel-callus formation, the data presented in Tables 6 and 10 indicate maxima about 39° and minima about 10° C. The optima are not so definite, for in general little difference was shown for any one series within the temperature range between 22° and 30°.

Although the gas and the water treatments employed in these experiments seem to have had little influence upon the cardinal temperatures, the index values obtained were markedly influenced by such treatments. Experiment 1 shows that at proper temperatures lenticel callus may form with a high partial pressure of oxygen surrounding the cuttings. Considering the low values of experiment 7 (flowing oxygen, bases of cuttings on stilts) in contrast with the high values of experiment 1 (flowing oxygen, damp cotton around bases of cuttings), the external supply of oxygen seems to have been of much less importance in lenticel-callus formation than was the water supply. This suggestion is supported by the difference in results shown between experiments 3 and 4 and between experiments 8 and 9. When experiments carried out at the same time are considered, lenticel callusing was increased in proportion to the quantity of water supplied to the cuttings.

However, considering the differences between experiments 5 and 6 (both without water treatment) lenticel-callus formation seems to have been favored by a low oxygen supply; in experiment 6, in which the cuttings received only 50 cubic centimeters per hour of a mixture of 5 per cent oxygen, index values were markedly higher than those of experiment 5, in which the same gas mixture was passed through the chambers approximately six times as fast.

Thus these willow cuttings subjected at adequate temperatures to negligible rates of evaporation showed formation of lenticel callus with any gas mixture tested, whether supplied with water or not; but such hypertrophy was accelerated either when the partial pressure of oxygen was low or when water was supplied to the cutting.

ADDITIONAL EXPERIMENTS ON ROOTING AND CALLUSING OF WILLOW CUTTINGS

Some additional experiments on rooting and callusing of willow cuttings were made, the numerical results of which are shown in Tables 8 and 9. Experiment 7-A (Table 8) was a duplicate of experiment 7 (Table 6) except that instead of having continuously flowing oxygen the chambers were swept out with oxygen at the start of the experiment, and this was repeated 12 hours later, after which the chambers remained sealed. Unlike experiment 7, this experiment shows about the usual minimum rooting temperature (15° C.) and the usual optimum (about 29°). But the maximum temperature (33.8°) is lower than that in any of the willow experiments with flowing gases. At this temperature actual injury was sustained because of oxygen deficiency. Although more oxygen was introduced into each chamber in experiment 7-A than was supplied in experiment 6 (slowly flowing 5 per cent oxygen), rusting of the wire stilts

took place rapidly, and the available supply probably fell below the critical limit for rooting. It is uncertain whether in experiment 7-A the generally low rooting values were all due to deficiency in oxygen, or whether under some temperature conditions an optimum supply was available. The occurrence of the generally low callus values here shown suggests that injury may possibly have occurred at the lower temperatures because of too much oxygen.

The four experiments, Nos. 13 to 16, were all run at the same time (February 26 to March 8); each chamber contained air when sealed, and (except in experiment 13) some preliminary water treatment had been given to the cuttings.

In experiment 13 the low values for rooting and the very high values for callusing are in accord with previous suggestions that callus formation is favored by a very low oxygen supply and that, although the required oxygen supply for rooting of willow is low, it is not nearly so low as is that for callusing in the same plant. In this experiment, at every temperature employed, except perhaps near the minimum, the supply of oxygen fell during the experiment period below that required for good root formation. At 28.7° and 32.8° C., temperatures which other experiments have indicated as being near the optimum for callusing, the oxygen supply fell even below the low amount required for optimum callus formation. Consequently, the temperatures here indicated for optimum callusing (20.3° to 23.7°) are much below the optimum (about 29°) indicated by experiments 1 to 12.

The results of experiment 13 show that, for the suboptimal temperatures employed, conditions were very good for callusing of willow; in fact, better than in any other of the writer's experiments. The fact that at a temperature of 12.2° C. moderate callus was here observed at both top and base indicates that the temperature minima (about 18° to 21°) shown by the experiments with flowing gases were too high. Apparently in every case in experiments 1 to 12 willow callusing was in general greatly reduced because of excess of oxygen.

Experiment 14 (in which the bases of the cuttings were soaked 24 hours) showed rooting values as low as or lower than those for experiment 13 at the corresponding temperatures, indicating for willow (as has already been observed for apple) that, with deficiency of oxygen, injury was even more pronounced with wet cuttings than with dry ones. In this case callusing at the bases of the cuttings (the region soaked for 24 hours) was almost negligible, while the values for top-callus formation are very high—almost as high as those for experiment 13. That the almost total absence of base callus was not entirely due to the 24-hour soaking seems indicated by the fairly high callusing values shown in experiment 9 (Table 6), where, with flowing air, cuttings whose bases had been soaked for 22 hours showed more callus formation at the base than at the top.

In experiment 15 (in which water was injected into the cuttings for 25 minutes) and experiment 16 (in which the cuttings were soaked one hour) the values for callusing are again low, and the values for rooting are even lower. The available oxygen supply here also probably fell much below the critical limit for optimum rooting and even somewhat below that required for optimum callusing. Injecting the cuttings seems to have decreased rooting and callusing slightly more than immersing them for one hour; in experiment 15 the maxi-

imum temperature for both rooting and callusing was below 33° C. It is probable that the lower callusing activity shown by these last three experiments was not entirely due to oxygen deficiency, for indications that treatment of the cuttings with water tends to retard callusing, irrespective of the effect of such treatment on oxygen supply, have already been referred to.

In Table 9 are shown the results of two experiments (Nos. 17 and 19) with gas not under continuous flow, which again indicate that a large supply of oxygen may retard rooting. In experiment 17 air and oxygen were used, with chambers A and C remaining closed throughout the 7-day period and with the gas bubbled through chambers B and D every 12 hours. The cuttings in chamber A (air closed) showed the highest rooting values of the four, those in chambers B (air, changed) and D (oxygen, changed) showing much lower rooting values. This experiment indicates that a large supply of oxygen was injurious, irrespective of the partial pressure employed. Experiment 19, A to D, also showed some indication that oxygen in excess may act injuriously upon the rooting of willow. Although the proportion of cuttings that rooted was not appreciably lower in chambers B and C than in chamber A, the lower average number of roots per cutting may have been due to higher oxygen content.

Table 9 also refers to many experiments with willow in which low rooting values seem clearly to have been due to deficiency of oxygen. In experiment 18, A to D, nitrogen, or a mixture of nitrogen and oxygen with 5 per cent of the latter, was used, no change of gas taking place in chambers A and C, but with the gas in chambers B and D changed every 12 hours. In all cases very low rooting values were obtained, but the highest value was for the chamber through which the largest amount of oxygen passed. In spite of the total amount of oxygen inserted in chamber B, it seems probable that, due to the rusting of the iron-wire stilts, an actual deficiency of oxygen occurred in this case as well as in the other three bottles where obviously very little oxygen was available for rooting.

In every experiment referred to in Table 9 (except experiment 17) one or more chambers showed evidence of oxygen deficiency. In some cases this deficiency was brought about by an insufficient volume of air, in some cases by inadequate partial pressure of oxygen in the gas mixture, and in some cases by a temperature too high for the other conditions.

The cuttings in experiment 23, chamber A, gave the highest rooting values obtained without flowing gases. The rooting values for the cuttings in chamber B and for those in chamber C are markedly different, which may well have been due to a difference in the available oxygen supply per cutting between the chamber that contained 4,200 c. c. of air at the beginning of the experiment and the chamber that contained 2,700 c. c.

The experimental results given in Table 9 appear to support the suggestion that callusing and rooting values do not reciprocate, but rather that each process is independent of the other, being influenced by water, oxygen, and temperature. Table 9 further indicates that callusing of willow was retarded (1) when water treatment was excessive (experiment 23), (2) when oxygen supply was too great (experiments 17 and 21), and (3) when oxygen supply was too small (experiments 18, 19, 20, and 23).

No clear indications regarding the controlling external factors for lenticel-callus formation were obtained in the experiments without flowing gases. Moderate lenticel callusing occurred whether the cuttings were supplied with large or with small amounts of water or oxygen. Only in experiment 18 (where all chambers had very little oxygen) did there appear any indication that oxygen supply was a limiting factor. Here all values for this form of activity were low, and apparently the lower the oxygen supply the lower were the values obtained. Also lenticel callusing seems to have had an even lower critical limit of oxygen supply than had top or base callusing.

A preliminary experiment set up October 26, 1926, indicated, however, that even with negligible amounts of oxygen very large lenticel calluses may form in time. In this experiment the bases of the cuttings rested on damp cotton, and nitrogen was bubbled through alkaline pyrogallol solution and then through the chamber containing the cuttings. A rubber bag with nitrogen under slightly more than atmospheric pressure remained attached to the chamber, the pyrogallol bottle being between the bag and the chamber. The set-up was placed in the greenhouse, shaded from intense sunlight. After one month the cuttings showed very large lenticel calluses, but rooting was almost negligible. Neither rooting nor callusing occurred during the next 12 days. On December 8 the chamber was opened to the air, and four days later many roots were visible on each cutting, indicating that lack of oxygen in the first part of the experiment had been the chief factor to retard rooting, even though optimum conditions had been offered for lenticel-callus formation.

Few indications regarding cardinal temperatures are to be obtained from Table 9, since only a single temperature was employed in each experiment. It may be noted, however, that the highest rooting values were shown by injected cuttings kept at 29° C., while the highest values for top and base callusing are shown by cuttings kept at 32° and supplied with additional water. In general, the higher the temperature the greater the supply of oxygen necessary for optimum rooting. This seems to have been indicated only slightly for callusing at top or base, and not at all for lenticel callusing.

In an experiment carried out in April, 1926, it was observed that one lot of willow cuttings with bases in water showed the same root development as did another lot with bases embedded in a 1.25 per cent agar gel. In the light of the more recent experiments, these results suggest that either the small amount of oxygen necessary for rooting was able to diffuse fast enough through the agar, or else the necessary oxygen reached the bases of the cuttings through the freely exposed tops. *Tradescantia* cuttings treated in the same manner as the willow cuttings also rooted in agar, but with this difference—the roots formed only near the top of the gel, suggesting that more oxygen is required for its rooting than for willow.

The experiments here reported with willow cuttings without flowing gases are no more than preliminary, but the results obtained seem to strengthen some of the suggestions brought out in experiments 1 to 12; namely, (1) that for both rooting and callusing a rather definite and comparatively low supply of oxygen is necessary; (2) that the minimum oxygen supply for callusing is less than that for rooting; (3) that excess of oxygen may slightly retard rooting and markedly retard callusing; and (4) that the oxygen minimum is

greatly dependent upon the temperature. The chief difference between apple and willow as shown in these experiments seems to lie in the much higher point for the oxygen minima in the former.

SUMMARY

The experiments here reported were of a general and exploratory nature, planned to secure indications as to promising methods of procedure in both the planning and the execution of tests of rooting and callusing. Fall, winter, and spring cuttings were tested in darkness, at seven different maintained temperatures in each of 16 series with constantly flowing gas, and these series differed with respect to the oxygen and water conditions in the test chambers. Some preliminary treatments with water were tested in this way, and some experiments were tried without flowing gas. With the different maintained temperatures and with flowing gas the experiment period was 10 days, while an experiment period of 7 days was used in some of the tests without flowing gas. The plants used were apple of the Springdale variety and willow (*Salix alba* L.), both of which have preformed, dormant root primordia under and in the bark. In the Springdale apple these primordia occur in groups, forming burrknots. The apple cuttings were made from wood from 3 to 10 years old; most of the willow cuttings were made from wood 2 years old.

RESULTS WITH APPLE CUTTINGS.—For the 10-day period the temperature curve of rooting showed generally a minimum of about 8° to 14° C., a maximum of about 39°, and an optimum of about 24° to 29°; but these cardinal points of the temperature relation were apparently shifted or modified according to the oxygen and water conditions.

The maximum temperature was consistently about 39° C. in all cases where the oxygen supply was adequate. When the oxygen supply was apparently inadequate, the maximum temperature was not so high.

The optimum temperature appeared to be influenced by internal conditions related to the seasonal maturity of the shoots from which the cuttings were made. With apparently adequate oxygen and water conditions the optimum for the spring cuttings seemed to be shifted downward on the temperature scale, and it was also markedly broadened into an optimal range. The fall cuttings showed a more definite and somewhat higher optimum than did the spring cuttings, and the latter rooted equally well throughout an extensive optimal range.

The numerical values or scores representing the amount of rooting were considerably greater for the optimal temperature range in the spring than they were for the fall optima. In the later tests, with more mature shoots, at sub-optimal and supra-optimal temperatures the rooting values were greater than at optimal temperatures in the fall tests. More rooting and rooting at lower temperatures generally occurred in cuttings made and tested in March than in those made and tested in November.

The minimum temperature for rooting appeared to be decidedly lower in the spring tests than in the fall tests, suggesting that the minimum was shifted downward on the temperature scale as the shoots became more mature.

The minimum temperature for rooting did not seem to be influenced by water and oxygen conditions as these were tested.

Some evidence secured suggested that a longer experiment period would have given lower minima than those given during the 10-day period.

While the slope of the temperature curve for rooting for supra-optimal temperatures appeared to be much influenced by oxygen and water conditions, this influence was not pronounced for sub-optimal temperatures.

Neither injurious nor beneficial effects on rooting appeared from exposing the cuttings to oxygen at a pressure of about 1 atmosphere. When ordinary air was passed through the test chamber at an adequate rate, the rooting in apple cuttings was just as satisfactory as when oxygen with only slight admixture of nitrogen was used.

Rooting values were higher when the cuttings had been subjected to an initial treatment with water, but the extent of such treatment might be excessive, in which case retardation was more or less pronounced, seemingly according to the de-

gree of excess. Thus, soaking the cuttings one hour in water resulted in increased rooting, with other conditions good, but soaking them 48 hours resulted in marked retardation of rooting. On the other hand, callusing seems to have been retarded by any water treatment, this retardation being approximately proportional to the degree of water treatment.

Callusing seems to have been more active at slightly higher temperatures and with somewhat lower water and oxygen supplies than those inducing the most active production of roots.

Although the maximum temperature for callusing was about the same as that for rooting and the optimum was only slightly above that for rooting, the minimum temperature for callusing was generally from 4° to 8° above the corresponding temperature for rooting in the same experiment.

With good environmental conditions, callus formation was generally found to be more active at the base than at the top of a cutting. This suggests an internal influence of polarity on callusing, in which respect callusing seems to differ from rooting, for which no such influence was apparent.

A distinct retardation of callusing appeared when the cuttings were surrounded with oxygen at a pressure of about 1 atmosphere, but no such retardation was observed in the case of rooting.

Although both rooting and callusing were more active at the season of the year when buds are ready for development (March), this increased activity in root and callus formation was as apparent for cuttings without buds as for those with buds; thus there seemed to be no direct influence exerted by buds on either rooting or callusing.

For formation of lenticel callus some oxygen seems to have been necessary, but the amount of oxygen required for lenticel callusing was much less than for top or base callusing.

RESULTS WITH WILLOW CUTTINGS.—In general, the results with willow cuttings were similar to those with apple, with some quantitative differences apparent in the cardinal temperatures and in the oxygen and water relations. The chief points of difference shown by willow cuttings were as follows:

Although for the rooting of willow cuttings an optimum temperature of about 29° C. was indicated, similar to that shown for apple, all temperatures tested between 20° and 33° were very good for willow.

Although some seasonal variation was observed, similar to that in apple cuttings, in all cases the minimum temperature appears to have been several degrees higher for rooting of willow.

All water treatments employed seemed beneficial to willow rooting; with a greater degree of water treatment, proportionally more rooting was apparent.

The minimum oxygen supply for rooting and callusing was so low that oxygen deficiency was not evident except in some of the experiments without flowing gases.

When no water treatment was used, a large oxygen supply seemed slightly to retard rooting. There was even some suggestion that the minimum temperature might be raised by oxygen excess.

Top and base callusing seems to have been more influenced in willow than in apple by excess of oxygen.

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OCCURRENCE OF THE ZONATE-EYESPOT FUNGUS *HELMINTHOSPORIUM GIGANTEUM* ON SOME ADDITIONAL GRASSES¹

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INTRODUCTION

In a previous paper³ the occurrence of the zonate-eyespot fungus (*Helminthosporium giganteum* H. and W.) on more than a score of grasses was recorded and a brief description of the lesions as they appear on each host was given. The account in question incorporated the results of observation begun in 1922, when the parasite was especially destructive and abundantly distributed, and continued as occasion offered not only in the vicinity of the District of Columbia but also in various other localities during the four following summers, all of which were marked by a considerably diminished prevalence of the fungus. The season of 1927 likewise revealed only moderate development of zonate eyespot, so that observations in the territory previously covered yielded little additional information. In 1928, however, the disease reappeared with more than ordinary severity. Its obvious destructiveness to a number of favorable hosts in the experimental grass plots at Arlington Experiment Farm, Rosslyn, Va., insured throughout August and September a liberal supply of conidia to which the other grasses under cultivation were exposed to a greater or less degree, depending, of course, somewhat on the relative distances and the positional features which the arrangement of the grounds entailed. Opportunity was thus afforded for noting especially the degree of susceptibility of a rather rich assortment of graminaceous species added to the plantings since 1922, among which many represent introductions from foreign lands where the parasite under consideration is not known to occur at present.

PRESENT INVESTIGATION

Foliar injury attributable to *Helminthosporium giganteum* was manifested in 11 grasses not hitherto recorded as hosts of that parasite. The determination of *H. giganteum* as the causal agent was accomplished through the identification either of fructifications growing out of the lesions or, in the case of the more unfavorable hosts, of the adhering evacuated conidial membrane, which from its position relative to the diseased tissue could safely be regarded as the envelope of the spore that had occasioned some particular instance of injury. It may be mentioned that two other species of *Helminthosporium* were present in large quantities in the experimental plots. One was the unnamed form briefly characterized in 1925³ as a parasite of redtop (*Agrostis palustris* Huds. = *A. alba* L.) but here found energetically

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² The writer gratefully acknowledges his indebtedness to Agnes Chase and A. S. Hitchcock for identification of various grasses discussed in the present paper and for information relating to nomenclatorial questions.

³ DRECHSLER, C. A LEAF-SPOT OF REDTOP CAUSED BY AN APPARENTLY UNDESCRIBED SPECIES OF *HELMINTHOSPORIUM*. (Abstract) *Phytopathology* 15: 51-52. 1925.

parasitic on seaside bent grass (*A. maritima* Lam.). The other caused severe leaf spot, mostly of the spot-blotch type, of a considerable number of grasses belonging to the genera *Bromus*, *Calamagrostis*, *Elymus*, *Festuca*, and *Lolium*, and would seem referable to *H. sativum* P. K. and B. Although in most cases the appearance of the lesion provided a valuable clue to the identity of the species of *Helminthosporium* involved, it could not be relied on sufficiently to obviate the necessity of procedure more trustworthy than macroscopic inspection. Thus a sprinkling of lesions of the simple eyespot type found on the leaves of *Calamagrostis epigeios* Roth, which strongly suggested injury from *H. giganteum*, was found attributable rather to the spot-blotch fungus. And again extensive foliar injury observed in a plot of buffalo grass (*Bulbilis dactyloides* (Nutt.) Raf.), which bore great resemblance to the zonate development of *H. giganteum*, could not be referred to any species of *Helminthosporium*, nor indeed to any other fungous agent, but was apparently of nonparasitic origin.

Among the various grasses severely attacked by *Helminthosporium sativum*, several bore evidence also of injury from *H. giganteum*, though the number and extent of the lesions due to the latter organism in such instances were for the most part inconsiderable. The pathological effects of the eyespot fungus on these grasses will not be discussed in the present paper, as it is hoped that in some other season a decreased development of the spot-blotch parasite, or preferably its absence, may make available a supply of less ambiguous material. In most of the plots of *Agrostis maritima* the presence of the sclerotium-forming species of *Helminthosporium* already referred to interposed a similar cause of confusion. However, in a few of the plantings, perhaps because of a greater degree of resistance in the host strain involved, infection from that source was present only in negligible quantity. Their close proximity to grass plots heavily infected with zonate eyespot, on the other hand, encouraged a fair representation of lesions due to *H. giganteum*, so that the reaction of seaside bent to this parasite could be observed under tolerably favorable conditions. The material used for illustrating the eyespot lesions on seaside bent, like that of the other hosts on which the presence of other parasites could be suspected, was first examined microscopically and the presence of an evacuated spore membrane of *H. giganteum* on each lesion confirmed. The likelihood of leaf spots other than those due to the zonate-eyespot fungus being included in the illustrations of this paper or in those of the preceding contribution, therefore, was effectively obviated.

In the following paragraphs the pathological effects appearing on the several grasses from exposure to *Helminthosporium giganteum* are briefly considered. Except when otherwise stated, the discussions are based on observations made at the Arlington Experiment Farm during August and September, 1928.

PATHOLOGICAL EFFECTS OF *HELMINTHOSPORIUM GIGANTEUM* ON VARIOUS GRASSES

In all plots velvet bent (*Agrostis canina* L.) was found very severely attacked by *Helminthosporium giganteum*. Because of the unusually narrow foliage characteristic of this host, the manifestation of injury is somewhat unlike that found in grasses with broad leaves. (Pl. 1, A-I.) On close inspection the incipient or small lesions, to be

sure, present the usual picture—a small, dead, bleached region surrounded by healthy green tissue. (Pl. 1, A, B.) With subsequent increase in extent as a result of secondary development, which very readily takes place under suitably moist conditions, the lesions soon occupy the entire width of the leaf. (Pl. 1, C-I.) As a result, a large proportion of the foliar organs in an actively growing stand exhibit dead segments of variable length alternating with living portions. Although in itself the destruction of segments of a leaf does not involve, at least immediately, the death of parts more distal in position, further extension of the fungus generally eventuates in the withering of the greater length of the blade affected. As the dead foliage is light-gray or light-straw colored, the general aspect of a growing turf in which the parasite has been operating for some time may be rather aptly compared to that of graying hair.

A very similar grizzly appearance is characteristic also of badly affected turf of susceptible creeping bent (*A. stolonifera* L.), especially when a narrow-leaf habit is encouraged through frequent mowing. A graying aspect is offered by diseased creeping bent even when cutting is infrequent; for, although on a close view the separate lesions can generally be distinguished on the somewhat broader leaves as discrete spots (pl. 1, T-Y; A'-J'), at a distance of 1 meter or more their individuality as well as that of the numerous completely withered leaves (pl. 1, Z) is much less apparent and thus becomes subordinate to the general appearance.

Information is not available as to whether the zonate eyespot is prevalent on velvet bent in the region, including especially the Northeastern States, where the grass is utilized in an economic sense both in ordinary lawns and more particularly as turf for golf courses. The section referred to is well north of the presumptive natural range of the disease, as far as the limits of that range may be surmised from the few collections which the writer has made. In this connection it may be pertinent to refer again to the discovery of *Helminthosporium giganteum* in various localities in Michigan, Minnesota, northern Ohio, northern Indiana, and northern Illinois, although no infection of other favorable hosts growing under ordinary natural conditions was found in those instances where a search was made. That the commercial distribution of infected stolons from sources within the natural range of the parasite may be held largely responsible for such northern occurrence can hardly be doubted. The distribution of velvet bent, however, has been almost wholly by seed, although a very limited traffic in material for vegetative propagation seems to have taken place in recent years. Because of its small size and the somewhat prolonged storage regularly entailed in commercial handling, bent seed would seem considerably inferior to stolons as a vehicle for the transmission of a fungus of apparently only ordinary hardness. The human agencies operating in favor of a wide artificial distribution of the parasite in the case of creeping bent may, therefore, not have been equally effective in the case of velvet bent.

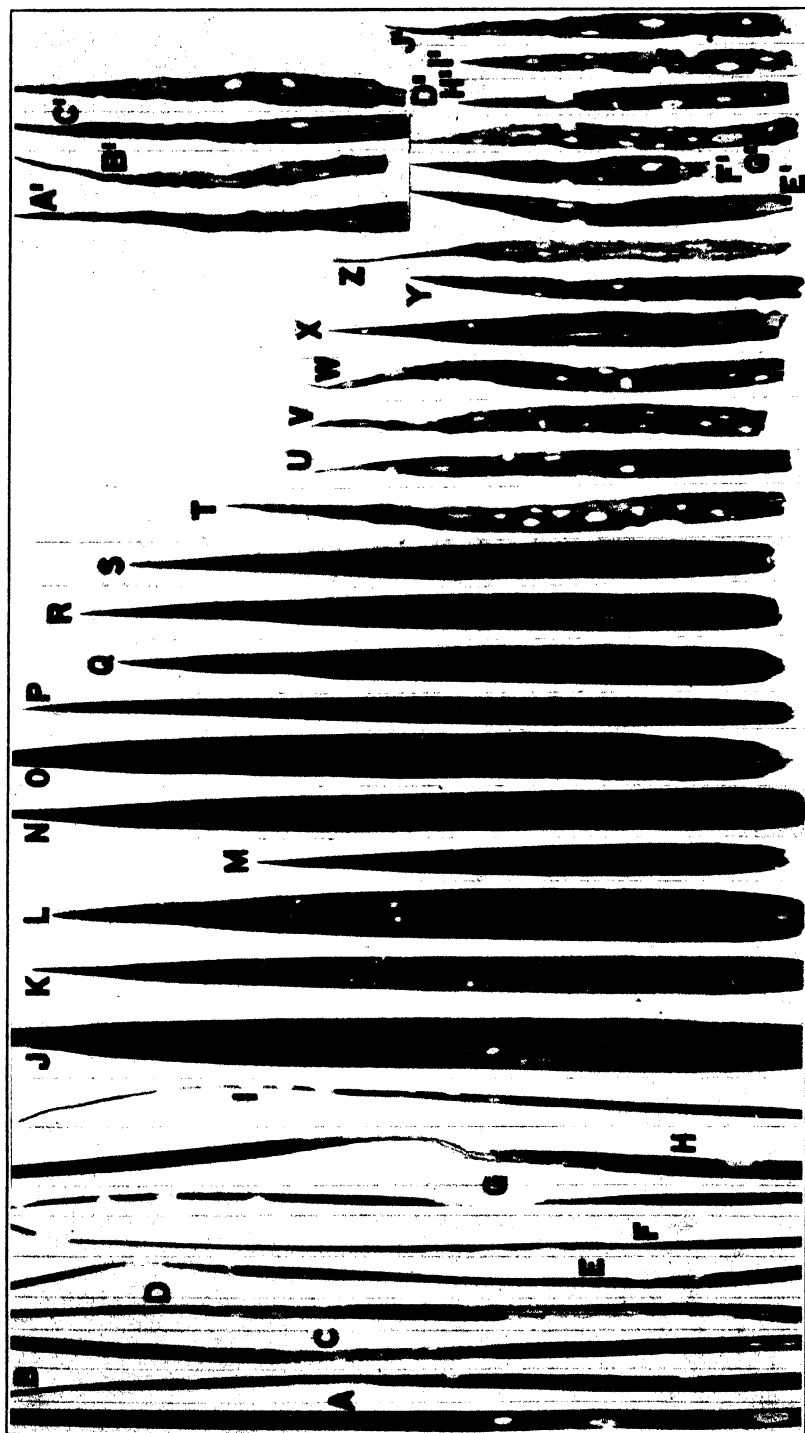
On the other hand, if the strains of velvet bent cultivated at Arlington Experiment Farm may be taken as representative of the species, this grass, once the parasite is established in a green, would suffer presumably quite as severely as the most susceptible selections of creeping bent. Irrespective of the kind of grass employed, the

conditions provided in putting greens, of course, are unusually favorable for the fungus. The frequent watering necessitated in the management of a satisfactory turf enables the parasite to extend its lesions and to continue producing conidiophores and conidia in quantity regardless of the intervention of droughty periods. Local distribution of conidia—a feature in respect to which the fungus would seem less successful under natural conditions than most of its graminicolous congeners—is undoubtedly promoted very materially by mechanical operations like mowing, rolling, or the removal of dew. Moreover, the extended season (from early in spring until late in fall) through which turf is kept in active vegetative condition can not but operate especially to the advantage of a pathogene somewhat slow in developing its attack.

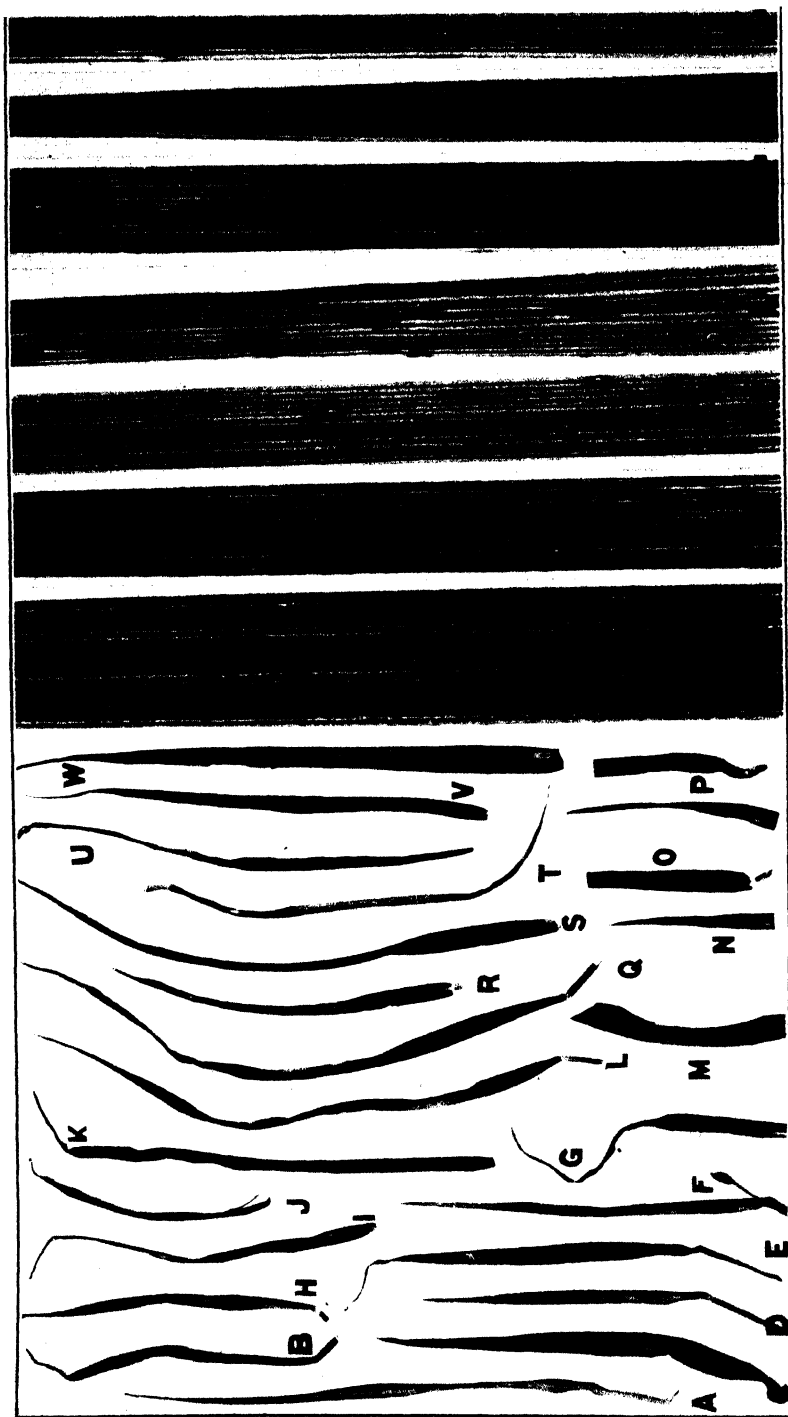
Agrostis maritima, growing in close proximity to very badly infected creeping bent, displayed only a relatively small number of lesions due to *Helminthosporium giganteum*. These lesions were of the simple eyespot type, relatively small, usually not measuring more than 0.5 mm. in length and 0.2 mm. in width, though occasionally attaining dimensions somewhat more than twice as great. (Pl. 1, J-S.) Even macroscopically the injury due to *H. giganteum* could usually be distinguished from spots attributable to the congeneric parasite so abundant on the same species of grass by their smaller size and especially by the relative proportion of central bleached tissue to the width of colored margin. In the main, the central areas of lesions due to *H. giganteum* were bleached rather completely, the contrast with the surrounding tissue being pronounced and the line of demarcation well defined, and the colored margin was comparatively narrow. On the other hand, the lesion caused by the other species of *Helminthosporium* consisted of a somewhat more vaguely defined, less completely bleached area surrounded usually by a wide zone of dull reddish coloration. It is hardly necessary to add that conidiophores of *H. giganteum* were never observed on the feebly attacked foliage of seaside bent, and that the latter, as far as present observations may be trusted, would not serve as host in the absence of other grasses.

Eragrostis caroliniana (Spreng.) Scribn. was found heavily attacked by *Helminthosporium giganteum*. (Pl. 2, A-W.) In the beginning individual infections were manifested in lesions of the eyespot type, which were often not more than 0.3 mm. in width and 0.5 mm. in length, and which had minute bleached centers delimited from the healthy tissue by moderately broad dark-brown or purplish-brown zones. (Pl. 2, E, K, N, O, R, W.) Subsequent enlargement of the diseased areas through centrifugal extension of the parasite occurred readily, not only bringing about malformations in younger leaves, traceable to locally impeded growth (pl. 2, B, C, M, S, L, T), but also eventually resulting in the complete or partial destruction of the foliar organ. (Pl. 2, B, G, I, L, P, Q, T, U.) Such destruction was apparent usually as a progressive withering of the affected leaf from tip toward base. As microscopic inspection of the dead tissue revealed an abundance of conidial fructifications of the parasite, there can be no doubt that *E. caroliniana* is to be reckoned among the more congenial hosts on which the parasite can maintain itself without difficulty.

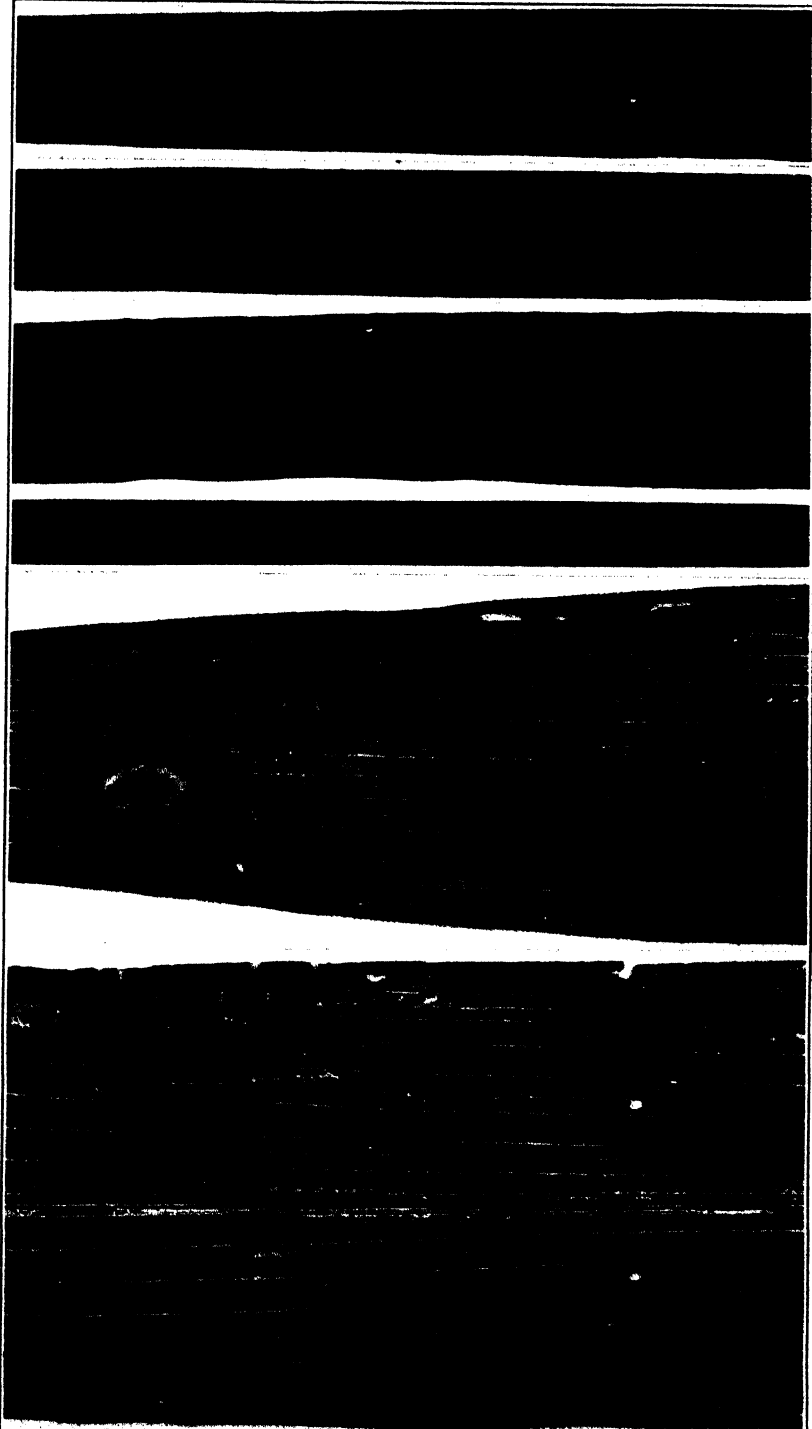
Festuca hookeriana (Benth.) Müll. (= *Schedonorus hookerianus* Benth.), a grass that plays a part in the agriculture of New South



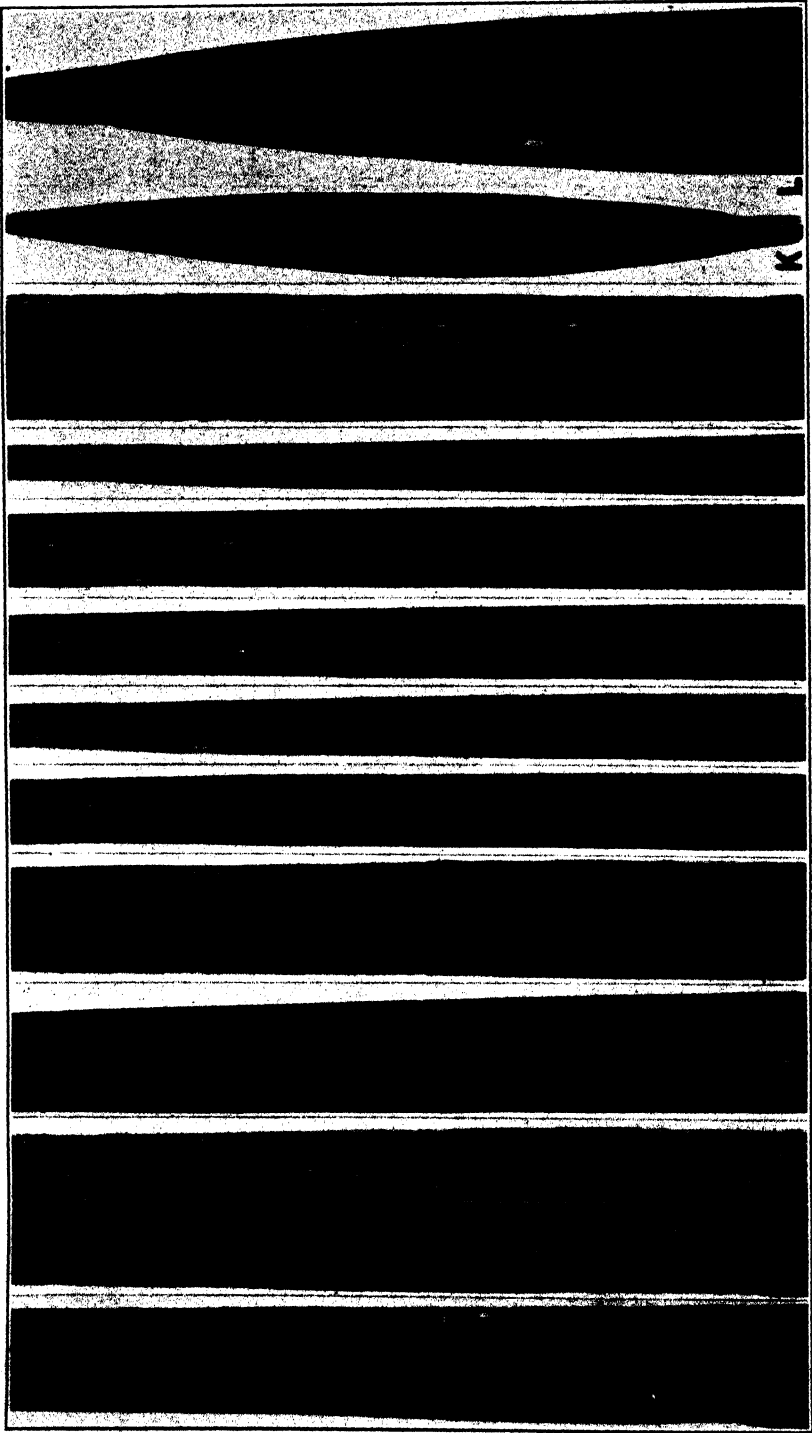
Leaves of grasses attacked by *Helminthosporium giganteum*, X 2: A-I, *Agrostis canina*; J-S, *A. maritima*; T-Z and A'-I', *A. stolonifera*



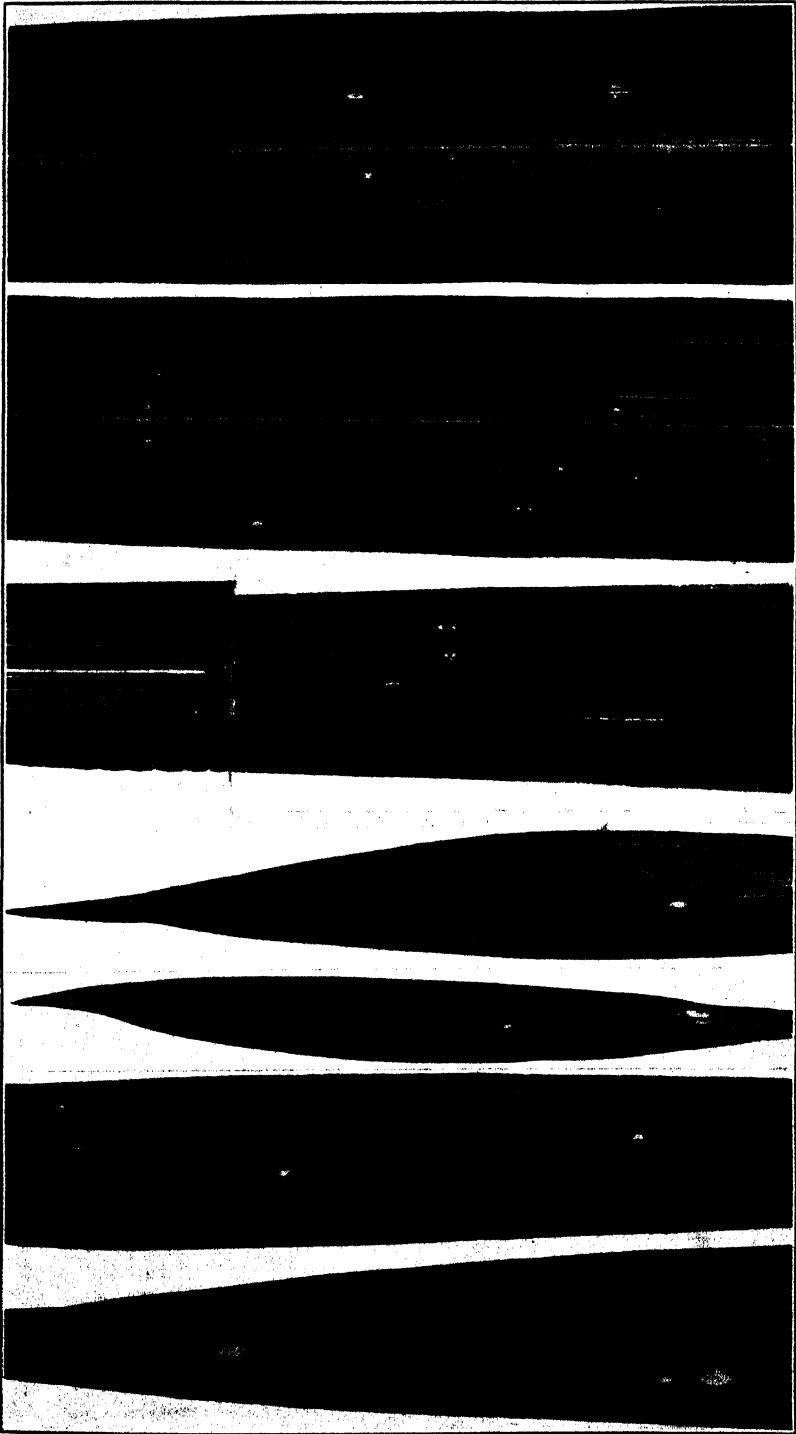
Leaves of grasses attacked by *Helminthosporium pigmentum*, X 2: A-W, *Fragaria caroliniana*; X-Z and A'-D', *Festuca hooleriana*



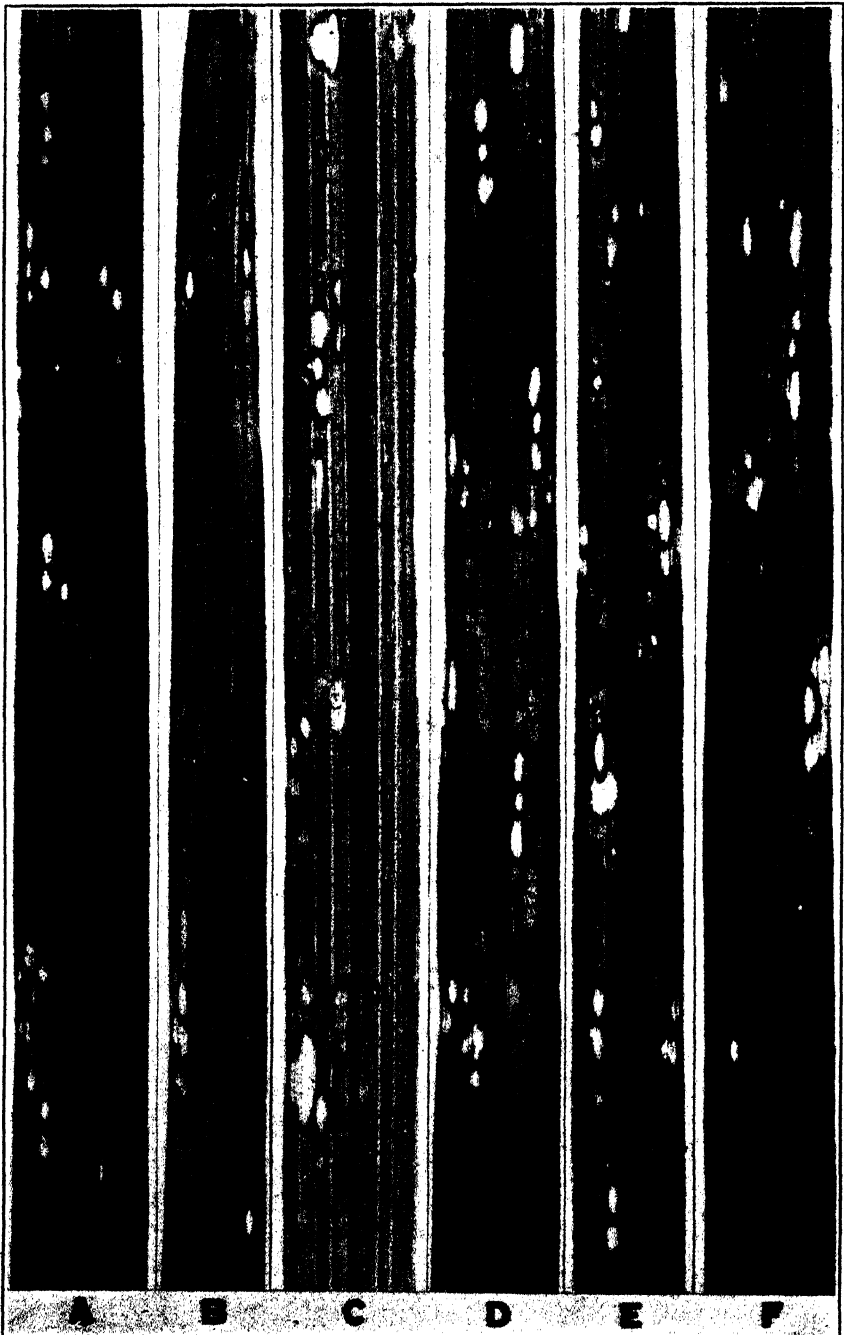
Leaves of grasses attacked by *Helminthosporium giganteum*, X 2; A and B, *Irophorus unisetus*; C, *Pennisetum ciliare*; D-F, *Phalaris bulbosa*



Leaves of grasses attacked by *Helminthosporium giganteum*, X 2: A and B, *Phalaris bulbosa*; C-J, *P. stenoptera*; K and L, *Spodiopogon sibiricus*



Leaves of grasses attacked by *Helminthosporium giganteum*, X 2: A-D, *Spodiopogon sibiricus*; E-II, *Tripsacum dactyloides*



Leaves of *Sporobolus* sp. (S. P. I. No. 75378) attacked by *Helminthosporium giganteum*, $\times 2$: A-D, series showing fresh peripheral infection in water-soaked stage present in increasing quantity; E, lesions abundant but with little zonal extension in progress; F, two zonate lesions with fresh peripheral increments; the other lesions show no current enlargement

Wales, Australia, somewhat comparable to that played in the United States by the brome grasses utilized here, was found spotted liberally with lesions due to attack by *Helminthosporium giganteum*. (Pl. 2, X-Z; A'-D'.) These were evident often as minute dark-brown spots sometimes not exceeding 0.1 mm. in width and 0.5 mm. in length. (Pl. 2, X, Y.) Occasionally the unrelieved eyespot type of discoloration persisted even in the largest of morbid regions with a width of approximately 1 mm. and a length of 4 mm. (Pl. 2, X, A', and D'.) More generally, however, the larger number of all except the smallest lesions revealed sharply delimited, somewhat bleached central areas, often so minute as to be barely discernible, but in instances of better development sometimes attaining widths of 0.5 mm. and lengths of 1 mm. (Pl. 2, Z, B' and C'.) In the resultant eyespot arrangement the dark-brown marginal zone was often, though not always, rather broad in comparison with the size of the central straw-colored portion. Distinctive zonate development was never observed, and microscopic examination uncovered no evidence of sporophores of *H. giganteum* arising from the discrete areas of affected tissue. It is hardly possible, therefore, that under ordinary conditions the grass would serve as a host of the parasite independently of other grasses. However, the abundance in which infection occurred would suggest that, under especially favorable conditions, with perhaps more extensive areas of morbid tissue resulting from the coalescence of separate lesions, the production of conidiophores and conidia might take place.

Ixophorus unisetus (Presl.) Schlecht, a native of Mexico, became marked in a somewhat unusual manner as a result of infection by *Helminthosporium giganteum*. The deep marginal discoloration evident in many hosts was represented on the upper aspect of the leaf by a blotch of purplish hue fading gradually into the green of the surrounding healthy tissue. In Plate 3, A and B, these purplish blotches are represented rather inadequately by the vaguely delimited dark regions surrounding the elliptical patterns, though their considerable extent is not inaccurately shown; the blotch exhibited near the upper left corner of Plate 3, B, for example, measures approximately 16 mm. in length and 7 mm. in width. The elliptical patterns consist, as the illustrations indicate, of outer zones of light coloration surrounding central regions of darker coloration, the former being of a light brownish hue and the latter of a darker reddish-brown or nearly brick-red color. The undersurface of the infected leaf generally exhibited no evidence of purple discoloration, but it did exhibit the reddish brown in the area corresponding to the central region, though usually in a slightly paler tone. The light-brown tone was represented on the undersurface only by a faintly colored halo separating the elliptical brown region from the green of the adjacent normal leaf tissue. In spite of the somewhat pronounced chromatic effects produced by the fungus, microscopic inspection failed to reveal any indication of conidiophores on any of the material examined. From the information available, therefore, *I. unisetus* can hardly be regarded as a sufficiently congenial host to propagate the fungus in the absence of other grasses.

On the leaves of *Pennisetum ciliare* (L.) Link were found a small number of longitudinally elongated dark-brown blotches attributable from microscopic examination to the germination of conidia of *Helminthosporium giganteum*. One of the largest of these lesions,

approximately 4 mm. in length and 1 mm. in width, is represented in Plate 3, C. Some of the blotches had bleached central parts, thus bringing about a somewhat poorly defined eyespot effect. Conidiophores were never observed. Evidently the grass under consideration, like the congeneric *P. alopecuroides* (L.) Spreng., is far too uncongenial as a host to support the parasite in the absence of more susceptible species.

Phalaris bulbosa L. bore a meager sprinkling of lesions, associated with overlying conidia of *Helminthosporium giganteum*. (Pl. 3, D-F and pl. 4, A, B.) Except for the incipient stages (pl. 4, B), the infections adhered to the eyespot type, a minute bleached speck being discernible often in examples not exceeding 0.7 mm. in length or 0.2 mm. in width. (Pl. 3, D, E.) In the larger examples measuring up to 2 mm. in length and 0.7 mm. in width, bleached center and dark-brown margin usually appeared in sharp contrast with each other and with the adjacent healthy tissue. (Pl. 3, F and pl. 4, A.) Conidiophores of the parasite were never observed on any of the affected leaves examined. However, the production of such structures could not well be expected from morbid tissue so inconsiderable in quantity. The independent propagation of the parasite on the host in question would seem altogether improbable.

A substantially identical reaction to the presence of *Helminthosporium giganteum* was observed on the congeneric and very similar *Phalaris stenoptera* Hackel. To be sure, an appreciably greater abundance of lesions is evident in the illustrations of the latter host (pl. 4, C-J) but this is to be attributed to the fact that the material utilized here consisted of leaves that had grown intermingled with badly infected foliage of *P. arundinacea* L. and had consequently been exposed most rigorously to conidia of the parasite. Indeed, if allowance is made for the obvious disparity in the severity of exposure, it is not certain that *P. stenoptera* might not be regarded as inherently of somewhat less rather than of greater susceptibility than *P. bulbosa*. In any case conidial fructifications of *H. giganteum* were as completely absent as in the latter species, and an approximately equal incapacity for the independent propagation of the fungus is indicated.

Spodipogon sibericus Trin. exhibited a relatively meager sprinkling of foliar lesions of the simple eyespot type attributable to infection by *Helminthosporium giganteum*. (Pl. 4, K, and pl. 5, A-D.) Bleached central parts were evident in some of the lesions that measured only 0.5 mm. in length and 0.2 mm. in width. (Pl. 5, A, B.) In the largest lesions observed, the dimensions of which were about six times as great, the elliptical cream-colored central areas were delimited rather sharply by the dark-brown or purplish-brown borders. (Pl. 4, L, and pl. 5, A, C, D.) In some instances a purplish discoloration appeared diffused in the tissue for some distance above and below the distinctly morbid part, a condition that may have been expressive of a certain measure of centrifugal infection. Under especially favorable conditions the host would seem to permit zonate development, although only to a slight extent, the halos surrounding the two larger lesions in Plate 5, A, and the grouping of the smaller lesions in Plate 5, B, being suggestive in this connection. However as microscopic examination failed to reveal presence of conidiophores on any of the affected leaves, *S. sibericus* should be considered among the more uncongenial hosts of *H. giganteum*.

An unidentified species of *Sporobolus* (S. P. I. No. 75378) recently introduced from Nairobi, Africa, by the Office of Foreign Plant Introduction, Bureau of Plant Industry, was revealed as one of the most favorable host of *Helminthosporium giganteum* hitherto encountered by the writer. The large number of individual lesions observed on the foliage provided evidence of a relatively low resistance to infection from germinating conidia (pl. 6, A-E), while an abundance of well-developed zonate patterns testified to the ready centrifugal extension of the parasite under suitable environmental conditions. (Pl. 6, A-F.) On microscopical inspection conidial fructifications of the parasite were found in quantity on the larger regions of killed tissue, indicating that once the parasite becomes established in a stand of this grass not only is the infection apt to become increasingly severe within the stand, but other grasses growing near by may be expected to become spotted in a measure commensurate with their proximity and inherent susceptibility. As the material used for the illustrations included in Plate 6 was collected at a time when rainy weather had prevailed during the preceding 16 hours, enlarging water-soaked zones are represented especially in Plate 6, D and F, in the somewhat poorly defined patches surrounding certain of the lesions. The more pronounced differentiation between cream-colored or straw-colored bleached areas and their delimiting dark-brown or occasionally purplish-brown borders becomes evident in this host, as in others, with the drying out of the affected tissue. The color values referred to are represented with fair approximation in the discrete lesions and older portions of zonate tracts shown in Plate 6.

Gama grass (*Tripsacum dactyloides* L.) was found very sparingly marked with incipient lesions attributable to *Helminthosporium giganteum*. (Pl. 5, E-H.) The morbid parts were manifested as brown elliptical spots, which were usually about 0.3 to 0.4 mm. in width and 1.5 mm. in length and surrounded by etiolated zones approximately 0.2 mm. in width. As might be expected in view of the inconsiderable mass of the tissue affected, the microscope revealed no sporophores of the parasite on any of the material examined. The rôle played by gama grass in the biology of the parasite, would seem, therefore, to be nearly negligible.

SUMMARY

Near Washington, D. C., 11 grasses not hitherto recorded as hosts of *Helminthosporium giganteum* were found naturally infected by it when growing under ordinary cultivation—that is, without artificial watering or other procedure that might accentuate the severity and prevalence of disease. Severe injury and abundant sporulation of the parasite was observed on velvet bent (*Agrostis canina*), a grass of some economic importance because of its usefulness in lawns and golf courses; on *Eragrostis caroliniana*, a common weed of no known value; and on an unidentified species of *Sporobolus* recently introduced from Nairobi, Africa, and of problematical economic utility. Readily noticeable though not severe injury, without sporulation, was observed on *Festuca hookeriana*, *Izophorus unisetus*, and *Spodiopogon sibericus*. On *Agrostis maritima*, *Pennisetum ciliare*, *Phalaris bulbosa*, *P. stenoptera*, and *Tripsacum dactyloides* only inconsiderable injury was detected, the lesions usually being both few and small.

MODIFICATION OF WESTERN YELLOW PINE ROOT SYSTEMS BY FERTILIZING THE SOIL AT DIFFERENT DEPTHS IN THE NURSERY¹

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INTRODUCTION

One of the most desirable characteristics in planting stock for use in the northern Rocky Mountains is drought hardiness. To this end it is generally considered necessary that stock should have relatively small tops and large bushy root systems, and consequently some cheap and effective means of producing stock of this description has long been sought. Underground root pruning (the cutting of seedling roots in situ) and transplanting in the nursery are the principal means thus far employed. These practices have, however, some disadvantages. Underground pruning alone might be effective if better spacing of seedlings could be attained by improvements in broadcast sowing or by sowing in drills. At present the spacing of plants in seed beds that have been sown broadcast is not sufficiently uniform to permit of root pruning in place without a heavy loss in dense patches of seedlings that do not have room enough for proper response to the pruning. Transplanting has an advantage over underground root pruning in that, in addition to determining proper root length, it results in a wider and more uniform spacing of the plants; but, on the other hand, it is a very expensive practice.

The possibility still remains that some other means, which can perhaps be combined with root pruning, may be found for regulating root growth. It should be considered that root length and the distribution of lateral rootlets may have as much influence on early drought hardiness as the general bushiness of roots. If, wherever planted trees are likely to suffer severely from drought, longer roots could be planted in deeper holes these trees would undoubtedly be much hardier. However, very long roots are not practicable, since they can not be planted in the field without doubling up in the planting hole, a practice which, while it might increase survival, might do so only at the expense of later healthy development. Root pruning before planting is therefore necessary. But another means of resisting the effects of drought is the use of plants that have a large portion of the total absorbing root surface in the lower half of the root system. This points to the desirability of finding some means of developing the root system between the depths of 4 and 8 inches below the surface of the ground. In seeking such means, the investigator naturally turns his attention to the influence of soil on root development.

The root systems of tree seedlings are not necessarily typical for the species, nor so controlled by hereditary tendencies as to be incapable of modification by environmental influences. Numerous inves-

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tigators have shown that at least five soil factors—moisture, fertility, physical properties, aeration, and temperature—influence the behavior of roots. Of these five, moisture and fertility are the most easily controlled in the nursery. Harris (10)² studying the behavior of corn, wheat, and peas, observed that an increase in root branching and a decrease in top-root ratio of plants was evident in the drier soils. Tucker and von Seelhorst (19), Jean and Weaver (11), and Weaver (21) in his study of the ecological relations of the roots of

native perennial plants on prairie lands, are in general agreement. Figure 1, taken from Weaver, shows a response of the root system strikingly similar to that obtained in the present author's experiments.

Carlson (6) and Weaver (21) are in general agreement that hard soil encourages root branching, whereas open soil appears to stimulate the growth of taproots. The importance of soil aeration was emphasized by Saussure (16) more than a century ago, and more recently by Snow (18), Clements (7), Barker (1), Cannon and Free (5), and Cannon (3, 4). Of the few who have contributed information regarding the influence of temperature, Engler (8) determined the lowest temperature for root growth of conifers to be 5° to 6° C., and Vesque (20) made observations on the effect of high temperatures on roots.

The fifth factor influencing root growth—fertility—is one that may be controlled in the nursery, and may be directly effective. It has been noticed that roots actually seek food, but that increased root branching caused by increased soil fertility may be accompanied by an increase in the top-root ratio of plants. Von Seelhorst (17) concluded that liberal fertilization resulted in larger and deeper root systems of rye, wheat, barley, peas, beans, and beets. Müller-Thurgau (13, 14) noted that nitrogen stim-

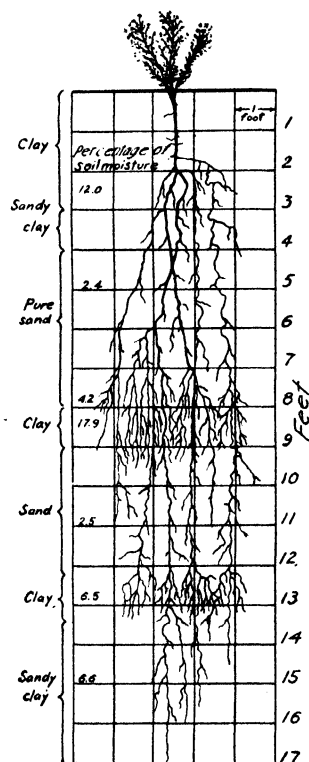


FIGURE 1.—Root system of *Kuhnia glutinosa*, after Weaver

ulated vigorous growth of secondary roots. Märcker and Kreusler (12, p. 94-101) and Nobbe (15) saturated alternate layers of soil with nutrients and after growing clover and corn, observed that the roots branched much more freely in the rich than in the poor soil. On the other hand, Harris (10) found that fertilizer decreased the root growth of wheat, possibly because soil nutrients may not affect all parts of root systems in the same way or to the same extent. Bates (2) stated that superabundant reproduction of forest trees, on sites that are poor from the standpoint of long-term forest production, may be induced because the seedlings, half-starved for soil nutrients, root deeply and safely.

In 1924 the possibility of modifying the root form of nursery-grown trees by means of fertilization became a subject of study at the

² Reference is made by number (italics) to "Literature cited," p. 145.

Savenac nursery, Haugan, Mont. In this paper the results of these experiments are reviewed and discussed.

EXPERIMENTS AT SAVENAC NURSERY

METHODS OF SOWING PLOTS AND RECORDING RESULTS

In these experiments on the regulation of root growth, three 4 by 12 foot beds were used, each divided crosswise by a central board partition into two plots of 24 square feet. The soil was excavated to

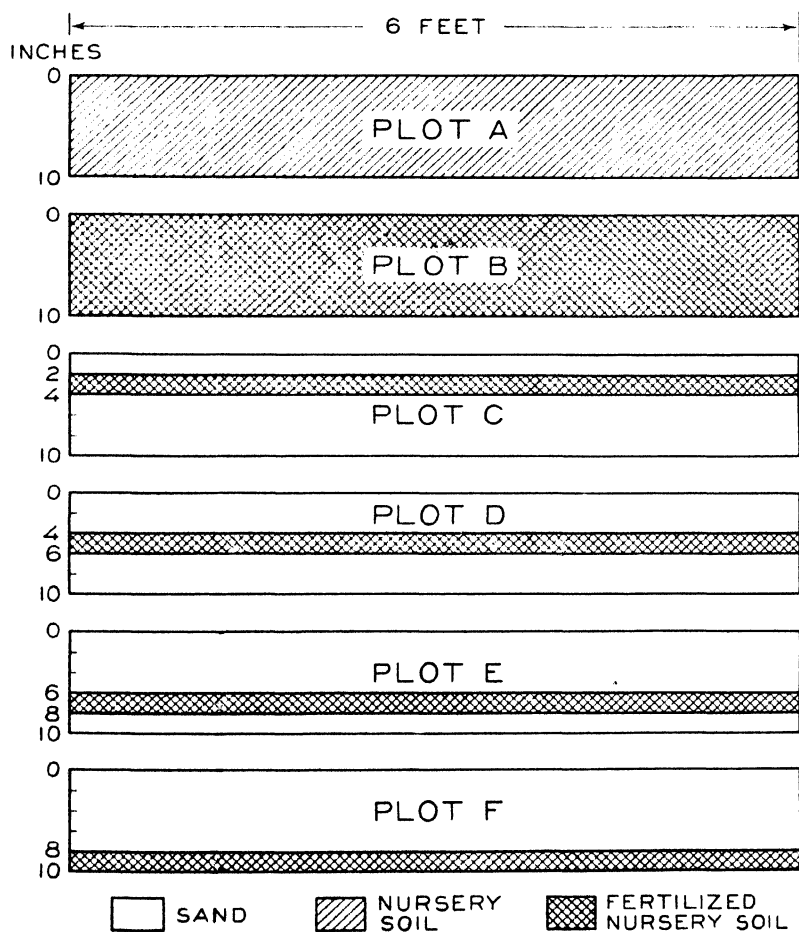


FIGURE 2.—Vertical longitudinal sections of experimental plots

a uniform depth of 10 inches, and excavations were filled with layers of sand and soil in the manner indicated in Figure 2.

The soil used in plot A was not sifted. All other soil was put through a screen having two meshes to the inch, except the small quantity used in constructing smooth sowing surfaces for plots A and B, which was sifted through a screen with three meshes to the inch. The finer of these two screens was used for sifting sand, except that

used in covering the sown seeds which was sifted through a screen of 0.1-inch mesh. These sifting processes not only provided more uniform media for root growth, but facilitated precise methods of constructing soil layers and sowing seeds. The depth of the various layers was accurately gaged by the use of special boards notched so as to slide on the lower horizontal side rails of the seed-bed frames.

The soil intended for each fertilized plot, including B, was in each instance thoroughly mixed in a box with 7½ pounds of sheep manure before placing in the plot. The fertilized soil was compacted in the measuring box by jarring and in the plots by tamping it into the desired space. Because the same quantity of fertilizer was used for plot B as for the others, concentration was less in plot B.

The sharp contrast in fertility of the 2-inch layers of manured soil with the surrounding sand was the condition sought. No measurements were made of the relative moisture-holding capacity of the two media, but samples of sand and unfertilized soil were put through sieves in order to show their difference in texture. The resulting figures given in Table 1 describe the soil materials more effectively than is possible in words.

TABLE 1.—*Mechanical soil analysis of representative samples of 407 grams of soil and 1,635 grams of sand*

Diameter of particles (millimeters)	Soil		Diameter of particles (millimeters)	Soil	
	Per cent	Per cent		Per cent	Per cent
Over 5.....	0.7	6.1	Less than ½.....	16.6	40.3
3 to 5.....	7.2	12.2	Lost in process.....	.3	.9
2 to 3.....	8.8	10.7			
1 to 2.....	22.3	16.5	Total.....	100.0	100.0
½ to 1.....	44.1	13.3			

The subsoil below 10 inches was not moved. It consisted of gravel, sand, and considerable clay in mixture and was quite dense; but the clay apparently contained more plant food than the pure sand used in the upper soil layers.

Sowing was done on May 13, 1924, with an allowance for each plot of 10 ounces of western yellow pine (*Pinus ponderosa*) seed collected in 1923 on the Bitterroot National Forest in western Montana. Sprinkling was frequently done during the spring and early summer, to bring out germination, but was less frequently done during the second season. Wire-screen frames successfully protected the beds from outside disturbance. By the end of the second season each plot contained a good stand of seedlings.

All trees to be removed from the plots for root study were first touched with a drop of white paint at the ground line. From a trench about 3 feet deep at the end of each plot a tunnel under the trees was started, care being taken to disturb none of the soil less than 14 inches from the surface. The soil was then washed down from the roots of the seedlings and into the trench with a garden hose. By this method of removing the trees root breakage was avoided. From each of the six lots a few trees that were much inferior in general development were culled out. Most of these were from hold-over germination and a year younger than their neighbors. In the laboratory the trees were fastened to a board ruled with horizontal

parallel lines 1 inch apart and were examined in detail one at a time. The painted ground-line of each tree was made to coincide with the uppermost line of the board. Roots were allowed to fall together in a loose mass as they would when planted, no attempt being made to spread out the lateral roots in the position in which they grew. All roots more than one-fourth inch long were counted each time they crossed a line, and the counts were recorded separately for each tree and line. Every 1-inch root segment to a depth of 14 inches was thus counted for 105 trees from each of the plots. Averages from these figures are given in Table 2, which forms the basis of the curves in Figure 3, the significance of which may be more readily seen from the graphs.

TABLE 2.—*Root frequency of 2-0 western yellow pines at different depths when grown in soil with fertilized layers at various depths*

[Each column gives the averages of counts made on 105 seedlings]

Distance from surface	Number of root sections in—						Distance from surface	Number of root sections in—					
	Plot A	Plot B	Plot C	Plot D	Plot E	Plot F		Plot A	Plot B	Plot C	Plot D	Plot E	Plot F
<i>Inches</i>							<i>Inches</i>						
1	1.1	1.0	1.1	1.0	1.1	1.0	9	5.7	3.5	3.0	3.1	7.0	8.3
2	2.8	2.8	3.0	2.1	2.6	2.3	10	5.2	2.9	2.3	2.4	5.2	9.1
3	5.4	5.8	6.3	3.8	4.8	4.0							
4	7.7	8.6	10.2	5.0	6.9	6.2							
5	8.5	8.9	11.1	8.2	8.0	7.4	11	4.9	2.7	3.5	3.0	6.3	9.1
6	8.4	7.6	7.6	8.9	7.6	7.9	12	3.2	2.0	3.0	2.3	6.4	8.9
7	7.7	6.0	5.2	6.5	9.5	7.8	13	2.3	1.7	2.6	1.3	5.1	7.6
8	6.5	4.8	3.9	4.4	9.6	7.8	14	1.6	1.5	2.2	1.2	3.2	5.6

Measurements were also made to determine the effect of the fertilization on the plant tops. The figures given in Table 3 show the tendency of fertilizer, especially in the deeper 2-inch layers of rich soil, to produce large tops.

COMPARISON OF RESULTS

In Figure 3 the curves for plots A and B may be taken to indicate the typical root development of 2-year-old western yellow pine seedlings. The general shape of the curves suggests the obconical, or inverted cone form, of which Haasis (9) speaks. The slight swelling in each of these curves below the 10-inch depth indicates a slight stimulation of root growth upon entering the subsoil. The average total mass of roots was greater on the trees from plot A than on the trees from plot B.

TABLE 3.—*Dimensions of western yellow pines grown in soils enriched at various depths below the surface*

[Figures are averages based on the measurement of 120 trees from each plot]

Plot	Treatment	Diameter at ground	Height from ground to tip of bud	Plot	Treatment	Diameter at ground	Height from ground to tip of bud
		<i>Mm.</i>	<i>Inches</i>			<i>Mm.</i>	<i>Inches</i>
A	Nursery soil.....	1.0	2.0	E	Rich layer 6 to 8 inches below surface.....	1.3	2.3
B	Fertilizer distributed.....	1.1	2.2	F	Rich layer 8 to 10 inches below surface.....	1.4	2.7
C	Rich layer 2 to 4 inches below surface.....	1.2	2.2				
D	Rich layer 4 to 6 inches below surface.....	1.1	2.1				

The tree roots from plot C form a similar curve with its greatest crest about 5 inches from the ground line, but these trees averaged 11 roots at that depth as compared with 8 or 9 on the same level in plots A and B, and root stimulation in the subsoil seems to have

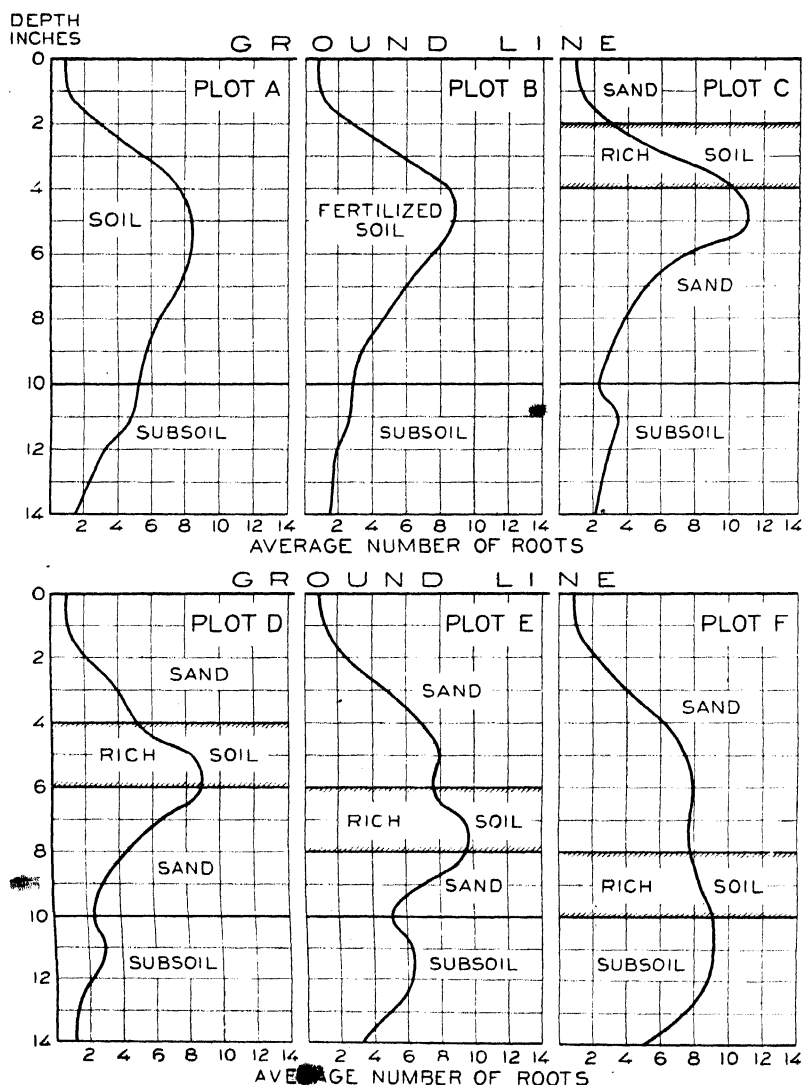


FIGURE 3.—Influence of soil fertility on the number of western yellow pine seedling roots at various depths. Each curve is based on the growth of fifty 2-0 plants

been greater in plot C. How these differences may be directly traced to the sharp contrasts in the soil medium will be made clear as the other curves are studied.

One question is natural here. If the increase in size and sharpness of the major crest in curve C is to be ascribed to the presence of the

2-inch layer of rich soil, why is it not centered in that layer 2 inches nearer the surface? It is not enough to note that the crests of the more natural curves A and B are at the same level. Three auxiliary reasons may be given: (1) Downward percolation of plant nutrients in the soil solution exceeded upward movement by capillarity, so that the zone around 5 inches deep became rich in plant food; (2) root growth that started under most favorable conditions in the rich layer continued downward into the 5-inch zone and affected the counts of root segments there; (3) roots that spread widely and approached the horizontal in the rich layer increased the root counts in the layer below when allowed to fall into more nearly vertical positions on the counting board. For these reasons it is found that the response of root growth to layers of rich soil are manifest in the curves in the lower part of, or just below, the layer of rich soil itself.

In the curve for plot D the major crest comes at the lower edge of the rich layer at the 6-inch depth. This is about an inch deeper than the natural crests of curves A and B, and hence the natural and artificial crests in curve D do not completely merge. This is indicated by the slight swelling at the 3-inch and the slight depression in the curve at the 4-inch depth. As in curve C, the response to subsoil in curve D is marked.

In the curve for plot E the position of the artificially produced crest bears much the same relation to the rich layer as in the other plots, but here it is low enough to be distinct from the natural crest, which shows again in its usual position 5 inches from the surface. The subsoil crest is present as before.

In plot F the rich layer was placed just above the subsoil. As the curve indicates, this position was so low as to cause the crest from rich soil to merge completely with that from the subsoil, so that the curve has two crests, the usual one above and the one produced by artificial means below, with a depression between at the 6 to 8 inch depth. Curves for plots E and F both indicate relatively large numbers of roots in the deeper layers of soil. Root increases in the subsoil in these plots appear to be largely due to branches which started growth in the rich soil not far above.

The above-mentioned characteristics of the root-frequency curves all seem to be consistent except one. If rich soil favors root branching and subsequent root growth, as is apparent in most of the plots, why should the root mass have been greater in the trees from unfertilized plot A than in those from fertilized plot B? The other soil factors known to influence roots—moisture, physical properties, aeration, and temperature—must have been much the same in the two plots, except as the small amount of fertilizer in plot B changed them. The fertilizer in plot B would make the soil slightly more retentive of moisture than that of plot A, thus tending to reduce the relative root growth of plot B, other things being equal. This reasoning may explain the behavior of the soil plots (A and B), but not that of the sand plots (C, D, E, and F). Possibly the cause of the inconsistency is a matter of general nutrition. The trees in soil grew in a natural medium with sufficient nutrient for ordinary needs, whereas the trees in sand were in a state of semistarvation and hence local root response was stimulated for the benefit of the plants as a whole. Unfortunately no plot was made of ordinary nursery soil containing a rich layer.

Whatever may be the true cause of this exception, the experiment seems to show the possibility of modifying root systems in the desired direction. The most desirable form of root system resulted from fertilized layers of soil 6 to 10 inches deep.

STUDIES IN FIELD PLANTATIONS

The extent to which the foregoing results obtained in the nursery affected the survival of trees planted in the field was tested. Two plantations each of western yellow pines from the six experimental plots were made on land typical of the burned-over areas in need of reforestation in the region. Just before planting all roots were pruned at a point 8 inches from the ground line, because longer roots could not be planted economically by the usual methods and with the customary tools. The first plantation was made in October, 1925, on a southeast slope with four hundred 2-year seedlings from each of the six plots. It was examined on August 25 of the following year. Although the plants were somewhat small for field use, by reason of the low average fertility of the seed beds and some crowding of seedlings, they served the purpose of the experiment.

In 1926 a similar plantation of 200 larger 3-year seedlings from each plot was made on a northwest slope, and was examined on September 16 of the following year—1927. The growing season of 1926 was one of drought; that of 1927 was moist. Thus, three conditions account for the generally higher survival of the second plantation, as shown in Table 4—the larger and better-developed seedlings, the more favorable site, and the more abundant moisture.

TABLE 4.—*Effect of modified root systems on the survival of western yellow pine seedlings one year after planting in the field*

[Based on a plantation of four hundred 2-0 and two hundred 3-0 seedlings from each of the six nursery plots. Average survival percentages were rounded off to the nearest whole number from basic figures carried to one decimal place]

2-0 SEEDLINGS PLANTED ON A SOUTHEAST SLOPE, OCTOBER 8 TO 12, 1925

Nursery plot	Depth of fertilization	Alive	Thrifty	Unthrifty
	<i>Inches</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
A	0	15	17	2
B	0 to 10	22	23	3
C	2 to 4	30	29	1
D	4 to 6	26	24	2
E	6 to 8	47	45	2
F	8 to 10	42	40	2

3-0 SEEDLINGS PLANTED ON A NORTHWEST SLOPE, OCTOBER 8 AND 9, 1926

A	0	81	74	7
B	0 to 10	77	69	8
C	2 to 4	74	67	7
D	4 to 6	78	72	6
E	6 to 8	92	89	3
F	8 to 10	90	86	4

The relative survival in the field of the trees from the series of six plots does not show consistent increases with increases in depth of fertilization, but when the figures are grouped in pairs, the average results show a tendency toward such an increase. In all cases the

percentage survival of the deeply fertilized trees, those exposed to a rich layer in the seed bed between 6 and 10 inches below the surface and having when planted a highly developed root system between 6 and 8 inches deep, was distinctly higher. This gives evidence of the actual superiority of such trees for planting.

CONCLUSION

From experiments at Savenac nursery in specially fertilized seed beds and from frequency curves showing relative root development according to depth of fertilizer in each bed, it has been found that the most desirable root forms of western yellow pine seedlings suitable for field planting occur in seed beds constructed with a layer of rich soil at a depth between 6 and 10 inches. Trees from such beds have highly developed root systems from 6 to 8 inches below the ground line and maintain the highest percentage of survival in field plantations.

The results clearly indicate the possibility, at least in perfectly controlled operations, of stimulating root development locally in any desired part of the root system by means of layers of rich soil. Burying fertilizers several inches deep may be expected to stimulate the growth of the lower root systems of seedlings by means of the greater contrast in zones of fertility, and at the same time to avoid certain undesirable effects near the surface of the soil such as unfavorable action on subsequent chemical treatments for the control of weeds or damping-off fungi, or interference with seed germination. But in this the operator must consider the condition of the subsoil. Deep fertilization may be wasteful whenever soil nutrients are rapidly leached out by downward percolation. At the Savenac nursery the subsoil is sufficiently impervious to prevent serious losses of this kind.

The results of the experiments herein described, although indicating certain possibilities, are at best only suggestive. Pending further experimental work and the perfection of root pruning in place and other improvements in seedling culture, the old and expensive process of transplanting will probably continue to be a necessary part of forest-nursery practice.

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THE USE OF DRESSED-BEEF APPRAISALS IN MEASURING THE MARKET DESIRABILITY OF BEEF CATTLE¹

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INTRODUCTION

The most direct and obvious way of measuring the market desirability of different lots of cattle is by the prices appraised or actually paid for the live cattle by representatives of the packing companies. There are, however, several serious objections to this method, the most important of which are the following:

(1) While the total sum paid for a shipment may very closely agree with its actual market value at the time of sale, the difference in the prices paid for individual lots in the shipment may not represent fairly the real difference in the market desirability of those lots. This is accentuated by two circumstances—the common practice of selling most of the cattle at prices which vary by 25 cents per hundred pounds and the usual bargaining psychology whereby a seller who thinks that the prices offered for all the lots are a little low finds it more expedient to insist on 25 or 50 cents more for one lot than to try to get 5 or 10 cents added to the price for all lots.

(2) The live sales price does not enable one to estimate the variation in the market desirability of different steers within the same lot. All the steers in a lot either sell at the same price or in some such manner as "12 head at \$8.75 with 3 out at \$7.50."

(3) The buyer and salesman are both estimating the kind of dressed meat which the steers will produce and the dressing percentages which will be obtained, and occasionally rather large mistakes are made on small lots.

(4) The price level changes from day to day, and the price of a lot marketed this month can not be compared directly with the price of another lot marketed a month earlier or a month later.

The object of the present paper is to describe and illustrate a simple, inexpensive method of expressing in a single figure the market desirability of a beef carcass. This, the carcass-appraisal method, was devised before the grading-chart method (now generally used in the nation-wide cooperative study of factors which influence the quality and palatability of meat) was proposed. The appraisal method appeared to be giving good results and was not abandoned when the grading method became available. On three different occasions both methods were used, and opportunities were thus provided to compare the two methods in a limited way.

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DESCRIPTION OF THE CARCASS-APPRAISAL METHOD

After the cattle are killed and the dressed beef has been in the cooler for 24 hours or more, men regularly employed by the packing company in selling meat to retailers are requested to estimate for each carcass the price which they think they could get for it in their regular sales territory. Each man makes these appraisals without consulting his associates. The carcasses are scattered about through the cooler in whatever order they are received from the killing floor. This means that they are not usually grouped together in exactly the same lots as the animals were fed. Consequently each carcass is appraised without direct reference to the other carcasses of the same lot.

On every occasion, except one shipment to Kansas City, Kans., at least three men were obtained for the appraising. The selection of men competent to appraise was made by an official of the packing company, usually the superintendent of the dressed-beef department. It was explained to this official that appraisers were wanted who had had long practical experience in the dressed-beef trade and who might be expected to recognize differences in the beef and to appraise those differences on about the same scale as the retail trade would. Since different classes of retail shops have different demands, there were usually included among the appraisers men who worked in different types of sales territory, so that the average appraisals might have a more general significance. For example, at Fort Worth, Tex., the appraising committee on several occasions consisted of one salesman whose territory was the city of Dallas, another who worked a car-route territory in northeastern Texas and southern Oklahoma, and a third who worked partly in Fort Worth and partly in car-route territory to the south and west. On several occasions the superintendent of the dressed-beef department himself acted as one of the appraisers.

Most of the appraisers had never participated in an affair of this kind and were diffident the first time about matching their judgment against that of other salesmen, especially when higher ranking officials of the packing company were present. However, it soon became evident that the estimates by the different salesmen were paralleling each other closely, and whereas on the first few occasions all, or nearly all, of the carcasses had been appraised in whole cents per pound, later many were appraised in half cents per pound, and on the last occasions many were appraised in quarter cents per pound. Statistical analysis of the data showed that the accuracy of the appraisals increased greatly with this increase in precision, and suggests that tenths or sixteenths of a cent would not be beyond the useful limit of the appraiser's ability to distinguish between the market desirability of different carcasses.

ACCURACY OF THE CARCASS-APPRAISAL METHOD

Table 1 shows the actual appraisals made for each of the first carcasses on which this method was tried, and Table 2 shows the calculations involved in measuring the accuracy of the appraisals. The method of calculation follows that given in the last two chapters of Fisher's *Statistical Methods for Research Workers*,² and is identical

² FISHER, R. A. *STATISTICAL METHODS FOR RESEARCH WORKERS*. 239 p., illus. Edinburgh and London. 1925.

with that used in a recent study of the accuracy of cattle weights.³ The term "degrees of freedom" is used to express the number of items which are really free to show variation in the data. Since variation is figured from the mean, which is itself a calculated figure derived from the actual data, one item is required to fix the mean, and there are really only 59 degrees of freedom in the 60 original appraisals. Since there were three appraisers, 2 degrees of freedom are involved in the variation caused by the appraisers not having exactly the same price levels in mind and, when the data are corrected for this difference in the price levels used by the appraisers there remain only 57 degrees of freedom in the data. Since there were 20 carcasses, 19 degrees of freedom are involved in the variation, caused by the fact that the carcasses were not exactly alike, and when the data are corrected for the differences between carcasses only 38 degrees of freedom remain on which to estimate the error of appraisal. The 60 items of original data give us only the same amount of information about the accuracy of appraisal that would have been gained had one man appraised 39 carcasses which were absolutely identical with one another. But since it would not be possible to find a group of identical carcasses, and there would have been no way of knowing by objective methods when two carcasses were identical, the error of appraisal has to be measured in this indirect way.

TABLE 1.—*Appraised values in cents per pound of dressed carcasses of check animals killed November, 1924, at Fort Worth, Tex., at the beginning of the first Kingsville experiment*

Breed and designation number of individual steers	Appraisal estimate of—			Average
	Salesman No. 1	Salesman No. 2	Salesman No. 3	
	Cents	Cents	Cents	Cents
Herefords:				
55.....	9	9	8	8 $\frac{2}{3}$
56.....	9	9	8	8 $\frac{2}{3}$
57.....	12	11	11	11 $\frac{1}{3}$
58.....	10	10	11	10 $\frac{1}{3}$
59.....	9	9	8	8 $\frac{2}{3}$
Hereford-Brahman crossbreds:				
45.....	9	9	8	8 $\frac{2}{3}$
46.....	8	8	8	8
47.....	9	8	8	8 $\frac{1}{3}$
48.....	10	10	9	9 $\frac{2}{3}$
49.....	12	11	10	11
Shorthorns:				
50.....	7	6	6	6 $\frac{1}{3}$
51.....	8 $\frac{1}{2}$	8	8	8 $\frac{1}{6}$
52.....	8	8	8	8
53.....	8	7	8	7 $\frac{2}{3}$
54.....	9	9	8	8 $\frac{2}{3}$
Shorthorn-Brahman crossbreds:				
60.....	9	9	10	9 $\frac{1}{3}$
61.....	8	8	8	8
62.....	8	8	8	8
63.....	9	10	10	9 $\frac{2}{3}$
64.....	8	9	8	8 $\frac{1}{3}$
Average.....	8.975	8.800	8.550	8.775

³ LUSH, J. L., CHRISTENSEN, F. W., WILSON, C. V., and BLACK, W. H. THE ACCURACY OF CATTLE WEIGHTS. Jour. Agr. Research 30: 551-580. 1928.

TABLE 2.—Calculations of the accuracy of the appraisals shown in Table 1

[Analysis of variance]

Cause of variation	Degrees of freedom ^a	Standard deviation (cents per pound)	Mean variance per degree of freedom	Total variance
All causes.....	59	1.237	1.5290	90.212
Difference in appraisers' price levels.....	2		.9125	1.825
Remainder.....	57	1.245	1.5506	88.387
Differences between steers.....	19		4.1428	78.712
Final remainder or error in appraisal ^a	38	.505	.2546	9.675

^a See text for explanation.

$$\text{Intraclass correlation} = \left(\frac{4.1428}{1.5506} - 1 \right) \frac{1}{2} = +0.836$$

The "error in appraisal" shown in the last line of Table 2 expresses the variation which would remain in the individual appraisal prices if the appraisers all had the same price level in mind and if the steers were so nearly alike that the average appraisal prices for all the steers were identical. In other words, it is a measure of how much these three salesmen disagreed among themselves. Perhaps an easier way to visualize this difference is to imagine that an infinite number of meat salesmen (of whom the actual three are a random sample) might appraise these carcasses with the same price level in mind. Then the appraisals of each carcass by each salesman would deviate at least slightly from the average of the infinite number of appraisals of that carcass. The figure, 0.505 cent per pound, in the last line of Table 2, is the standard deviation of the difference between one salesman's appraisal of a carcass and the average appraisal of the same carcass by an infinite number of similar salesmen. It may also be considered as the standard deviation of one salesman's appraisals on a group of carcasses which would all have equal average values if appraised by an infinite number of salesmen. In short, it is the standard error of appraisal. It is itself a compound figure measuring both the differences between the ideals of different salesmen and the inability of the same salesmen always to set exactly the same price on carcasses which are identically alike. Which of these two sources of variation is the more important is not revealed by these data.

The intraclass correlation may be considered as a sort of average figure expressing the correlation between the prices set by different salesmen on the same carcasses. It does not measure in actual units the accuracy of the appraisal because it will be higher on groups where there are large differences between the carcasses than on groups where the carcasses show very small differences, even though the appraisers can come within the same number of cents per pound of agreeing with each other in both cases. The intraclass correlation is, however, useful in comparing the unanimity of opinion among the salesmen making the appraisals and the unanimity of opinion among the men using the grading-chart method when both groups of men work on the same carcasses.

One other point requires explanation. The data in Table 1 are treated in Table 2 as if all the steers belonged to a single lot. Each lot or breed might have been treated by itself. The effect of such a treatment would have been to bring all three appraisers to the same

price level for each of the four lots instead of merely bringing them to the same price level for all four lots combined. This would not sensibly have changed the figure for the accuracy of the appraisals *unless* the ideals of the appraisers differed in regard to the desirability of the type of carcass associated with an entire group. With cattle of such distinct types as Herefords or Shorthorns and the Brahman crossbreds, such a difference of opinion was likely to occur. Consequently on every occasion the data were analyzed both lot by lot and as a total regardless of lot divisions. The lot-by-lot analysis eliminated any prejudice for or against an entire lot which one salesman might have had but which was not shared by the other salesmen. The analysis of the totals includes such a prejudice as part of the error of appraisal. The former viewpoint would be most nearly correct for such studies as differences between steers in the same lot, correlations of individual prices within a lot with other measurable characteristics such as weight, previous gain, etc. The accuracy based upon totals seems the most suitable for use in comparing differences between lots or between individual steers belonging to different lots. A lot-by-lot analysis of the data in Table 1 is shown in Table. 3.

TABLE 3.—*A lot-by-lot analysis of the data given in Table 1*

Group	Standard deviation of error in appraising	Intra-class correlation
Herefords	0.548	+0.827
Hereford-Brahmans	.428	+.885
Shorthorns	.408	+.811
Shorthorn-Brahmans	.428	+.750
Average	.456	

* See text for explanation.

The average standard deviation was obtained by squaring each standard deviation, multiplying these squares by the number of degrees of freedom on which each is based, adding, dividing the sum by the number of degrees of freedom, and extracting the square root of the quotient. This method was followed wherever standard deviations were averaged in this paper. In this particular case there is no conclusive evidence that these appraisers held a different attitude toward the different types of carcasses for, while the figure 0.456 is actually lower than the figure 0.505, the difference does not even approach statistical significance. However, in the grand average of all appraisals on all occasions, this difference, while still of about the same magnitude, does become statistically significant because it is based on numbers so much larger. Thus there is evidence that, on many occasions at least, the appraisers differed significantly among themselves in regard to the relative value of a certain type of carcass characteristic of an entire lot of steers. This of course is not surprising in view of the fact that on every occasion there was at least one lot carrying three-eighths or more of Brahman blood and at least one lot quite free from Brahman blood. In the usual feeding experiment where all the lots are equally divided with respect to

breed and sex, such a difference in the attitude of the appraisers toward certain lots would probably not be encountered.

These appraisals were made on nine different occasions and in three different packing plants. In all, 42 lots of cattle differing in breed or in method of feeding and including a total of 429 steers, were appraised. Besides the steers fed during three years at Kingsville, Tex., the steers used in two years of feeding experiments at Spur, Tex., were included. The weighted average standard deviation of the appraisals for all cattle (after eliminating differences in price level and differences between carcasses) was as follows:

- (1) Analysis of each lot of steers separately (based on 786 degrees of freedom)—0.421 cent per pound.
- (2) Analysis when all steers slaughtered at one date are regarded as a single group (based on 848 degrees of freedom)—0.467 cent per pound.

The difference between these two figures, although small, is quite significant statistically and is the evidence previously mentioned that on some occasions, at least, the appraisers were not agreed on how to value a type of carcass characteristic of an entire lot of steers.

There was much variation in the size of this standard deviation, which ranged from as high as 0.77 cent in one case to as low as 0.11 cent in another. The only very evident cause of this variation was the degree of precision with which the appraisers worked. The evidence on this point is given in Table 4. If even a single appraisal was given in half cents (as was the case in Table 1) the whole group of appraisals on that occasion was classified as being in half cents. Thus even among the appraisals classified as being made in quarter cents, an unduly large number were given in whole cents or half cents and distinctly less than half of them were given in quarter cents. With this in mind it should be clear from Table 4 that the salesmen were still far from reaching the limits of their appraising ability when they made appraisals in quarters of a cent. In order to get approximately full value out of data, the grouping interval should be at least four times as large as the standard deviation. Even with the finest degree of precision used here, the grouping interval (quarters of a cent) is almost as large as the standard deviation of the error of the appraisal. Apparently these salesmen were so well agreed as to what makes a carcass desirable or undesirable to the trade that the data would have been still more valuable had they appraised the carcasses with a still finer degree of precision; for example, to tenths or sixteenths of a cent per pound.

TABLE 4.—*The relation between precision of appraisals and the size of the errors in appraising*

Precision of appraisal	Degrees of freedom	Standard deviation of error in appraising (cents per pound).	
		Range	Average
In whole cents:			
Lot-by-lot analysis.....	52	0.53-0.75	0.656
Analysis of total.....	58	.77	.767
In half cents:			
Lot-by-lot analysis.....	350	.28-.62	.508
Analysis of total.....	379	.42-.67	.550
In quarter cents:			
Lot-by-lot analysis.....	384	.11-.43	.265
Analysis of total.....	411	.24-.36	.297

COMPARISON OF THE CARCASS-APPRAISAL METHOD WITH THE GRADING-CHART METHOD

On three occasions when 14 lots of cattle totaling 171 head were used the carcasses were not only appraised as described above but were also scored by the grading committee working on the national cooperative project "factors influencing the quality and palatability of meat." Two of these three occasions were during the first year of this grading committee's work, when the methods were still being developed and some of the members of the committee were still somewhat inexperienced in the procedure of grading carcasses. The results must therefore be considered as extremely tentative. The comparison is made at this time because these three occasions are the only ones known to the writers on which both methods were used and there is no definite prospect of further data of this kind becoming available.

In the carcass grading the several members of the committee worked independently, as did the packer salesmen in making their appraisals. The graders examined each carcass both in general and in detail and graded it with respect to each of 40 different characters or points. Conformation, finish, and quality were the general characteristics considered. Compactness, thickness of flesh and external fat, marbling, texture and color of lean, conformation and finish of loin, rib, chuck, rump, and round, and other details were graded. Seven grades—prime, choice, good, medium, common, cutter, and low cutter—with three subgrades in each, were recognized. The total score, representing the grade and subgrade of the carcass, was obtained by adding the numerical values corresponding to the gradings of the 40 points. The committee grading of the carcass was represented by the average of the total scores of the different members of the committee. This method of judging the market desirability of beef carcasses has the distinct advantage, from the research standpoint, of involving consideration of the inherent causes of the differences and a record of the causes.

The standard deviation of the error in the total score for each carcass, calculated in the same way as described above, was—

Lot-by-lot analysis, based on 361 degrees of freedom—1.71 per cent.

Analysis of totals, based on 386 degrees of freedom—1.80 per cent.

The difference between these two figures suggests that the grading committee, like the salesmen, could not always agree on how to rate a type of carcass characteristic of an entire lot of steers. The difference in this case, however, is smaller and not surely significant, since a difference as large or larger than this could happen by chance alone somewhat oftener than once in twenty times.

Since an average error of 0.421 cent can not be compared directly with an average error of 1.71 per cent, the direct comparison of the degree of agreement among the appraisers and of the degree of agreement in the grading committee was made by comparing the intraclass correlations on the same groups of steers. Such a comparison is shown in Table 5. The average correlations presented in Table 5 are simple unweighted arithmetical averages of the intraclass correlations shown there, and probably should be a little higher because large correlations ought to receive more weight than small ones in a

highly accurate method of averaging and also should be weighted in some manner according to the numbers involved. The intraclass correlations shown were calculated exactly as shown in Table 2 without correction for any bias caused by the smallness of numbers. If properly corrected for this bias these correlations would each be slightly higher than the uncorrected values actually shown.

TABLE 5.—*Comparison of the degree of agreement in the grading committee and the degree of agreement among the packer salesmen*

Group of cattle	Carcasses	Graders	Intra-class correlation of grading committee's carcass grades	Salesmen	Intra-class correlation of salesmen's prices on meat
	<i>Number</i>	<i>Number</i>		<i>Number</i>	
Second Kingsville:					
A, March slaughter—					
Lot 1.....	6	4	+ 0.780	3	+ 0.860
Lot 2.....	8	4	+ .424	3	+ .777
Lot 3.....	8	4	+ .916	3	+ .680
Lot 4.....	9	4	+ .563	3	+ .731
All steers as a single lot.....	31	4	+ .638	3	+ .800
B, April slaughter—					
Lot 1.....	15	3	+ .708	2	+ .609
Lot 2.....	15	3	+ .846	2	+ .816
Lot 3 ^a	13	3	+ .697	2	+ .119
Lot 4.....	10	3	+ .862	2	+ .862
All steers as a single lot.....	53	3	+ .750	2	+ .541
Third Kingsville:					
Nos. 1-13.....	13	3	+ .799	3	+ .871
Nos. 14-25.....	11	3	+ .864	3	+ .943
Nos. 26-50.....	24	3	+ .793	3	+ .873
Nos. 51-75 ^b	14	3	+ .645	3	+ .595
Nos. 76-88.....	13	3	+ .783	3	+ .805
Nos. 89-100.....	12	3	+ .759	3	+ .915
All steers as a single lot.....	87	3	+ .765	3	+ .875
Unweighted arithmetical averages of—					
14 single lots each calculated separately.....	171	4 and 3	+ .746	3 and 2	+ .747
3 slaughtering occasions, each calculated as a single group.....	171	4 and 3	+ .731	3 and 2	+ .742

^a 1 carcass in this lot was not graded and therefore was also omitted from the price correlation.

^b 10 carcasses from this lot were graded but were shipped before they could be appraised. Therefore they are also omitted from the correlation of the grades.

It will be seen that on the first occasion the correlation was higher for the salesmen than for the grading committee on the total and on three of the four lots. This was the occasion on which the grading committee had had least experience. On the second occasion the correlation was higher for the grading committee than for the salesmen on the total and on three of the lots and was the same on the fourth lot. The second occasion was the first and only time the price appraisals were secured at a Kansas City plant, and only two men participated. The correlation between their appraisals was distinctly lower than most of the correlations, although there were scattered cases of correlations lower than this for single lots on other occasions. On the third occasion the correlation was higher for the salesmen on the total and on five of the six lots.

From a study of the figures in Table 5 it may be concluded that there was no great difference in the degree of agreement among the

salesmen and that among the members of the grading committee, since the average for the correlations of prices and the correlations of grades are almost identical.

DEFECTS OF THE CARCASS-APPRAISAL METHOD

The appraisal method has some distinct disadvantages, among which are the following:

(1) The price is given for the carcass as a whole and it is therefore impossible to determine from the data whether a low price is due to overweight, insufficient fatness, dark color, "staggy" conformation, or other causes.

(2) The price level in the dressed-beef market is constantly changing, and prices appraised at one time may not be directly comparable with prices appraised a month earlier or a month later. This difficulty could of course be overcome in large part by transforming the appraised prices into some kind of index numbers based on the general price level of the dressed-beef market, but even then there would remain some question as to whether the appraisers were exactly in step with market changes or whether they lagged behind a little.

(3) The price level is slightly different in different markets for different classes of beef. For example, heavy beef is quite unpopular in the Southwest for all except a very limited hotel and club trade, but is in much greater demand in the industrial regions of the Northeast. Moreover, fatness does not usually carry as large a premium in the South as it does in the Northeast. Consequently a lot of heavy fat steers probably would not receive as great a premium over a lot of slightly thinner, much smaller steers if appraised by salesmen familiar only with the country-trade territory around Fort Worth as they would if appraised in Jersey City, N. J., by salesmen familiar with that trade territory.

In view of these defects the appraisal method can not, of course, be recommended where one desires to determine the reasons for a carcass being desirable or undesirable. As a method of describing the details of the individual carcasses the method is useless, but where one wants merely a measure of the desirability or undesirability of the carcass as a whole the appraisal method has the advantage of being inexpensive and reasonably accurate. It also has the advantage (for some purposes) of being readily translatable into differences between the prices of live steers, and hence of permitting, on a somewhat sounder basis than heretofore, the estimation of profits or losses from different methods of breeding, management, or feeding. Both the appraisal method and the grading-chart method possess the very important advantage of enabling the investigator to measure the variation within lots and thus to make an estimate of whether differences in sale prices are significant or accidental.

TRANSLATION OF APPRAISAL PRICES INTO LIVE-WEIGHT PRICES

The simplest method of translating the dressed-meat prices into live-weight prices consists in multiplying the average appraised price for each lot by the number of pounds of chilled beef produced from that lot and dividing the product by the sales weight of the corresponding live animals, or (which is the same thing) multiplying the average appraised price per pound by the commercial dressing per

cent. The figure thus obtained would, however, express only the value of the meat per pound of live weight. With beef cattle the by-products are so important that the beef often sells for less than the live-cattle cost. According to Clemen,⁴ meat furnishes only 87.3 per cent of the packer's total revenue from steers, hides furnish 8.6 per cent, and by-products furnish the remaining 4.1 per cent. The problem is further complicated by the fact that costs and profits or losses of the packing company vary not only from plant to plant but from day to day and from lot to lot on the same day. Add to this the fact that the appraised prices for the meat may not actually be realized in the final sale, and it becomes clearly evident that there can be no entirely accurate method of determining what the packer should have paid for the entire shipment in order to obtain a given percentage of profit. It is possible, however, to estimate with a fair degree of accuracy what the differences should have been between the prices paid for the different lots in any one shipment.

Moreover, it is often desirable to express prices, which are based on meat values, at about the same price level as the prices actually paid for the cattle. This can be accomplished roughly by several methods. The simplest (hereafter called method A) is to add to (or subtract from) the meat value per pound of live weight for each lot a constant figure of such size that the unweighted average price for all lots would be the same as the unweighted average price actually paid. In Table 6 are shown the data for four lots of steers marketed in May, 1925, from an experiment at the King ranch, Kingsville, Tex. The meat values average 69 cents per hundred pounds of live weight less than the actual sales prices were. Consequently the corrected sales prices shown under method A are obtained by adding 69 cents to the meat value for each lot. This correction has the effect of making the corrected sales prices average the same as the actual sales prices but adjusts the differences between lots to conform to what was found out later about dressing percentages and about the appraised values of the dressed meat. According to the correction, it appears that the buyer slightly underestimated the price for the Hereford lot and the Shorthorn-Brahman lot or overestimated the price for the other two lots. The difference is not large nor was it so in most cases, but occasionally there were lots on which the buyer seemed to go rather far astray. Doubtless the prices at which the live animals are bought or appraised would be more accurate if one could get averages of prices appraised independently by several buyers and salesmen.

TABLE 6.—*Methods of translating dressed-meat prices into live-weight prices*

Item	Prices of—			
	Herefords	Hereford-Brahmans	Shorthorn-Brahmans	Shorthorns
Average dressed-meat prices..... per hundredweight..	\$15. 11	\$14. 23	\$13. 38	\$14. 89
Commercial dressing percentage.....	57. 2	59. 4	61. 4	56. 6
Meat value of live weight..... per hundredweight..	\$8. 64	\$8. 45	\$8. 22	\$8. 43
Actual sales price..... do.....	9. 25	9. 25	8. 75	9. 25
Corrected sales price:				
Method A..... do.....	9. 33	9. 14	8. 90	9. 12
Method B..... do.....	9. 33	9. 15	8. 90	9. 11

⁴ CLEMEN, R. A. BY-PRODUCTS IN THE PACKING INDUSTRY. 410 p., illus. Chicago. [1927.]

In method B, which is more complicated, the total meat value of the shipment is made equal to the total net proceeds actually received by the shipper. The necessary total correction is divided among the total carcass values for the different lots in proportion to those total values. In method B the correction is a proportion of the total values per hundred pounds of live weight and is most extreme for the lots having the highest meat value per pound of live weight, while in method A the correction is a flat amount and is the same for all lots. Which method has the more logical basis depends upon whether the buyer of the live cattle makes his mistakes as a general rule on a percentage basis or on a flat basis. For example, if the buyer is just as apt to make a half-cent mistake on an 8-cent lot as he is on a 16-cent lot, then method A is the more accurate. On the other hand, if 1-cent mistake on the 16-cent lot is just as likely to occur as a half-cent mistake on an 8-cent lot, then method B is more accurate.

In determining the general level of the corrected prices method A gives equal weight to all lots whether they contain few steers or many, while method B gives weight to the different lots according to the price and weight of the live cattle in the lot. Method B is thus to be preferred from the accountant's standpoint. Method A would be preferred by the research worker who regards each lot as a representative sample from a very much larger population and conceives it to be only an incidental matter that there were only a few steers in one lot and many in another.

In most cases the two methods agree very closely, but the agreement is unusually close in the example used in Table 6. Both methods are very rough approximations and must compromise on many points. For instance, in obtaining the average dressed-meat price all carcasses in a lot are given equal weight. From the point of view of an accountant it might be more desirable to give more importance to the heavy carcasses and less to the light ones. Such weighting of the average dressed-meat prices was not done in this study. The probable errors of the corrected sales prices shown in Table 7 were obtained simply by multiplying the average commercial dressing percentage of the lot and the probable error of the average dressed-meat prices. Besides that part of the error of appraisal which is not eliminated by using the average of three independent appraisals these probable errors also include the sampling error arising from the fact that the carcasses are not all alike in any lot. This sampling error is much more important than the error of appraisal in nearly all lots included in these studies. The probable errors shown in Table 7 are only very rough approximations, as, for most purposes, one would want also to take into account the additional sampling error arising from variation in dressing percentages within each lot. Nor is the fact taken into consideration that the lots are carefully balanced according to the best judgment of the investigator when the experiment begins. A portion of the variation is thus foreseen and discounted before the experiment begins and should not be included in the real probable error, if there were a reliable method of measuring it. However, if we regard differences of less than twice their probable errors as not requiring explanation and differences of more than four times their probable errors as almost certain to mean a real difference in the market desirability of the two lots, we shall probably not be led very far astray. Certainly we would have a clearer understanding of the

situation than if we used only the actual sales prices and hence had no approximation at all of the probable error of those prices.

TABLE 7.—Comparison of prices actually paid and prices corrected to conform to appraised carcass prices

Designation of cattle	Prices per 100 pounds live weight		
	Actually paid	Corrected by—	
		Method A	Method B
Kingsville, first year:	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>
At beginning of experiment—			
Herefords	4.75	5.07±0.21	5.00
Hereford-Brahmans	5.00	4.90±.21	4.87
Shorthorn-Brahmans	4.75	4.55±.13	4.59
Shorthorns	4.00	3.98±.14	4.10
After 120 days of feeding—			
Herefords	8.25	9.08±.05	9.03
Hereford-Brahmans	9.00	8.73±.08	8.71
Shorthorn-Brahmans	8.50	7.99±.10	8.02
Shorthorns	7.75	7.69±.19	7.78
After 179 days of feeding—			
Herefords	9.25	9.33±.04	9.33
Hereford-Brahmans	9.25	9.14±.05	9.15
Shorthorn-Brahmans	8.75	8.90±.04	8.90
Shorthorns	9.25	9.12±.05	9.11
Kingsville, second year:			
At beginning of experiment—			
Herefords	6.00	5.70±.18	5.69
Shorthorn-Brahmans (first cross)	5.00	4.47±.18	4.51
Shorthorn-Brahmans, F ₂	6.82	7.53±.19	7.47
Shorthorns	5.95	6.08±.21	6.07
After 120 days of feeding—			
Herefords	9.35	9.45±.11	9.46
Shorthorn-Brahmans (first cross)	9.15	8.04±.07	8.99
Shorthorn-Brahmans, F ₂	8.85	9.26±.07	9.28
Shorthorns	9.60	9.30±.05	9.31
After 150 days of feeding—			
Herefords	9.00	8.67±.04	8.67
Shorthorn-Brahmans (first cross)	8.50	8.55±.03	8.56
Shorthorn-Brahmans, F ₂	8.50	8.65±.05	8.66
Shorthorns	8.50	8.64±.04	8.64
Kingsville, third year:			
Lot 1, Herefords and Shorthorns	9.00	8.76±.07	8.75
Lot 2, Shorthorn-Brahmans	9.00	9.11±.05	9.10
Lot 3, Shorthorn-Brahmans	9.00	9.20±.05	9.19
Lot 4, Herefords and Shorthorns	8.50	8.43±.04	8.43
Spur, 1924-25:			
3-year-olds—			
Herefords	8.88	8.62±.05	8.62
Hereford-Brahmans	9.00	8.82±.09	8.81
2-year-olds—			
Herefords	7.50	7.95±.05	7.99
Hereford-Brahmans (station bred)	8.50	8.11±.18	8.14
Hereford-Brahmans (purchased)	8.50	8.89±.18	8.88
Spur, 1925-26:			
2-year-olds—			
Herefords	9.50	9.75±.03	9.70
Hereford-Brahmans (first cross)	9.50	9.75±.05	9.70
Back crosses	9.50	9.57±.04	9.52
Yearlings—			
Herefords	10.00	9.89±.03	9.84
Hereford-Brahmans (first cross)	10.00	9.82±.03	9.78
Back crosses	10.00	9.73±.04	9.68
Spur, 1926-27:			
Yearlings—			
Herefords	9.50	9.61±.05	9.58
Hereford-Brahmans (first cross)	9.96	9.65±.06	9.62
Back crosses	10.00	9.61±.06	9.58
Swenson grade Herefords fed—			
Ground milo heads	9.65	10.20±.07	10.19
Entire ration ground	9.73	9.95±.06	9.94
Entire ration unground	9.74	9.50±.07	9.54

Considering the amount of approximation involved in thus translating dressed-meat prices back into live-weight prices, the question may well be raised whether such translation is worth while. In the writers' opinion it is worth while for popular presentation because it combines in one figure the effects of differences in price per pound and differences in dressing percentage. Such translation may not be necessary in technical publications, for the readers of these can keep in mind the separate consequences of dressing percentages and desirability of meat. However, in their studies of Brahman cattle, the writers have usually had to deal with cases where the higher dressing percentages are associated with the lower values per pound of the dressed meat, and in such cases it is not always easy to see at a glance which combination of factors results in the greater market desirability of the live animal.

Table 7 presents the actual and the corrected prices for all the shipments made during three years of cattle-feeding experiments at Kingsville and at Spur, Tex. The probable errors shown for method A might with equal justification have been shown for method B, but in the latter case they have been omitted to prevent the table from becoming needlessly complicated.

Even though the probable errors given in Table 7 are only rough approximations, it seems evident that differences of less than 15 or 20 cents per hundred pounds of live weight can rarely be considered as significant unless they occur again and again each time the experiment is repeated. And yet differences smaller than these have frequently determined the relative profitableness of two lots in published reports of feeding experiments.

In surveying the whole group of data in Table 7 one can not avoid being impressed by the closeness of agreement between the actual prices paid by the buyer for the live animals and the prices which dressing percentages and subsequent appraisal of the dressed meat showed. This general rule has its striking exceptions, however, as in the case of the second shipment during the first year of feeding at Kingsville, where the Hereford lot was very distinctly undervalued. On the contrary, in the final shipment during the second year at Kingsville, the Hereford lot was distinctively overvalued by the buyers. Among the 1925-26 steers from Spur, the 2-year-olds were undervalued and the yearlings were overvalued by the buyer. The previous year the buyers had favored the older cattle slightly more than the younger ones.

A casual survey of the data does not reveal any consistent penalizing of any age or type of cattle, beyond what the appraisal values of the beef justify. The nearest approach to this is in the second year of Kingsville feeding where the double-cross (F_2) Shorthorn-Brahmans were bought at less than their meat value would justify at all three marketings. During the first year at Kingsville, Brahman cattle were overvalued five times and undervalued once. During the second year they were overvalued twice and undervalued four times. During the third year they were undervalued both times. During three years' marketing from Spur, the Brahman lots were overvalued six times and undervalued three times. In all, 13 of the 23 lots containing Brahman blood were overvalued and 10 were undervalued.

The buyer's judgment can not be disregarded entirely, and in cases where he disagrees markedly with the dressed-beef appraisals

it should probably be assumed that the true value lies between the two estimates but much nearer to the estimate of the carcass appraisers. It is concluded from this study that in the carcass-appraisal method we have an inexpensive and much more refined weapon for attacking the problems of the relative market desirability of different lots of cattle than when we merely accepted the sales price as correct. The carcass-appraisal method eliminates the guessing about dressing percentages and how the carcass will appear when dressed. It measures the variation in the desirability of different carcasses in the same lot and thus permits an estimation of the significance of differences between the market desirability of various lots. It seems worthy of much more confidence than the actual live-weight selling price, but the latter should not be entirely ignored.

THE MAGNITUDE OF ERRORS IN LIVE-WEIGHT PRICES

The question is frequently raised whether it would be commercially practical or desirable to sell beef on the basis of dressed weights and grades and thus to eliminate the guessing about dressing percentages and about how the meat will appear when dressed. If such a commercial practice would insure that each individual producer would be paid more nearly on the basis of the quality of the cattle he sells than he is paid at present, it would to that extent be desirable. The data presented herewith have some bearing on this question, inasmuch as they throw light on the accuracy of carcass appraising and grading. They also throw light indirectly on the question of the accuracy of the live-weight prices.

If it could be assumed that the prices given in Table 7 under method A were absolutely correct, then it would develop that the mistakes in the live-weight prices ranged from -83 to +53 cents per hundred pounds. If we give equal weight to every lot in Table 7 there will be 45 differences between the live-weight prices and the prices corrected according to method A, but only 35 degrees of freedom, since the corrected prices were so calculated that their average had to equal the average of the actual prices for each of the 10 marketing occasions. The standard deviation of those differences is 34.7 cents per hundred pounds where method A is used as the standard and 33.8 cents where method B is used. But it can not be assumed that the prices arrived at by either method A or method B are absolutely correct. They are subject to a slight standard error of appraisal which theoretically varies inversely with the square root of the number of steers in the lot and the number of appraisers, and which is of about the magnitude of 3 to 4 cents per hundred pounds of live weight on lots of 8 to 12 steers where three men do the appraising. (The error of random sampling among the steers would not be included here.) The standard deviations of 34.7 and 33.8 cents mentioned above are therefore standard deviations of differences and would be reduced slightly if the error of appraisal in the corrected prices could be eliminated. This reduction would be extremely slight, however, if the error of appraisal is anywhere near as low as has been indicated. At all events the standard error of appraising in the live-weight sales price seems to be in these data approximately 30 cents per hundred pounds of live weight or perhaps a little more. Expressed more concretely, this means that if these

data constitute a fair sample of market practice, the buyer buys half of the lots of cattle at within about 20 cents per hundred pounds of live weight of the price which they are really worth. Conversely, half of them are bought at more than 20 cents above or more than 20 cents below their real value on that market. About 5 per cent of them are bought at more than 60 cents too high or more than 60 cents too low. This is done unintentionally and with no desire on the part of the buyer to discriminate against any type or age of cattle. It results solely from the fact that it is impossible for the buyer always to estimate correctly the dressing percentage and the appearance of the dressed meat from live cattle.⁵

SUMMARY

Salesmen employed by packing companies show a high degree of agreement as to what constitutes desirable beef in the eyes of the retail butcher. The standard error of the appraising of a single carcass by one salesman, the market level being constant, ranged from as low as 11.2 to as high as 75.1 cents per hundred pounds of dressed meat on single lots and from as low as 24.4 to as high as 76.7 cents per hundred pounds of dressed meat where an entire shipment of several lots was studied as a single lot. The weighted average of all these standard deviations was 42.1 cents where only a single lot is considered and 46.7 cents where an entire shipment is considered.

A more detailed analysis of these figures shows that these average standard deviations are reduced to 26.5 cents and 29.7 cents, respectively, when we include only those shipments on which the salesmen made appraisals in quarters of a cent per pound. The data suggest strongly that salesmen can appraise meat with a reasonable degree of accuracy to at least as fine a degree of precision as tenths or sixteenths of a cent per pound.

With the usual experimental lot of 10 steers, and with three salesmen appraising independently with a degree of precision at least as fine as quarters of a cent per pound, the findings of this study indicate that the probable error of the accuracy of the average appraisal of the lot would be about 3.4 cents per hundred pounds of dressed meat on the single-lot basis and about 3.7 cents per hundred pounds of dressed meat on the basis of the entire shipment. The appraisal method would therefore be accurate enough to detect differences larger than 10 to 15 cents per hundred pounds in the average value of the dressed meat from two experimental lots of the usual size, if each lot were perfectly uniform.

Additional variation between the carcasses of each lot will nearly always be important enough to require differences greater than 25 cents per hundred pounds of dressed meat in order to demonstrate a

⁵ Since the above was written, there have come to the authors' attention data bearing on this same problem in the following publication: EYVARD, J. M., CULBERTSON, C. C., WALLACE, Q. W., and HAMMOND, W. E. ROUGHAGES FOR FATTENING TWO-YEAR OLD STEERS. Iowa Agr. Expt. Sta. Bul. 253, p. [388]-422, illus., 1928. Only seven lots of steers were included. Each lot was credited with the value of the hides, fats, and other by-products produced by it. An average loss to the packer of 46 cents per hundred pounds of live weight was computed from the figures, but this varied from as low as 14 cents for one lot to as high as 83 cents for another. The deviations of these losses from their average were +38, -15, -81, -23, 0, -5, and -8 cents per hundred pounds. Three of the seven deviations exceed 20 cents and the average deviation (neglecting signs) is 17 cents. In view of the small number of lots included, the authors of the present paper regard these Iowa data as quite in agreement with their conclusion that half of the lots of experimental cattle are valued alive at a price more than 20 cents above or more than 20 cents below their real value even if the average price of the entire shipment is assumed to be correct.

significant difference between two lots. Experimental lots so uniform that smaller differences than this would be significant can be imagined, but it is doubtful whether they often occur. Unless the lot is extremely uniform the error of the appraisal is probably of minor importance compared to the variation among the carcasses.

The salesman showed more agreement with each other than the members of the grading committee did on two occasions and less on a third occasion. The unweighted arithmetical averages of all comparable intraclass correlations were almost identical for the appraisal method and for the grading-chart method. The evidence indicates that the salesmen agreed as closely with each other on what constitutes desirable beef as did the members of the grading committee. In view of the relative inexperience of the grading committee when these data were taken, it is reasonable to suppose that subsequent data would show a still closer agreement between the members of the committee.

The appraisal method does not permit analysis to determine why appraisal values are high or low. For this object the grading chart used by the grading committee was especially designed.

The appraisal method is inexpensive, and the writers found the salesmen quite ready to cooperate in the appraisals after they thoroughly understood the purpose of them.

By the appraisal method it is possible to express the results on the basis of live-weight prices. For such translation several methods may be used. Each involves some assumptions which make the translated prices only approximate.

A comparison of the translated prices with the actual sales prices reveals a rather close general correspondence, but occasional instances occur where the actual sales prices were far from corresponding to the value of the meat. Such unjustified sales prices seem to arise from errors in the salesmen's or buyers' judgment and not from any consistent prejudice on the part of the buyer for or against any type, age, or breed of cattle. The standard deviation of the differences between the prices actually paid and the corrected prices is 34.7 cents per hundred pounds when method A is used for correcting and 33.8 cents when method B is used. After making allowance for the small error of appraising in methods A and B, it appears that the standard error of appraising in the actual live-sales price of experimental lots of steers is about 30 cents per hundred pounds of live weight. For comparison with other experimental lots in the same shipment, buyers are within about 20 cents per hundred pounds of the correct price for half of all their purchases. Errors greater than 60 cents per hundred pounds of live weight occur in only about 5 per cent of the lots.

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A STUDY OF DECAY IN CITRUS FRUITS PRODUCED BY INOCULATIONS WITH KNOWN MIXTURES OF FUNGI AT DIFFERENT CONSTANT TEMPERATURES¹

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INTRODUCTION

In making a survey of the forms of decay occurring naturally in the field and packing house one is impressed by the fact that the symptomatology of the various forms of rotting under such conditions presents a somewhat different picture from that of the decays induced artificially by inoculations with single pure cultures in the laboratory.

Since the inoculation of fruit with known mixtures of different species of fungi appeared to be a somewhat unexplored field (the majority of investigators having followed the classic path of single pure-culture inoculations), and since it was a method more nearly approximating the natural conditions for infections in the field, a series of differently combined inoculations as well as inoculations with the same organisms singly has been tried for the purpose of obtaining a better understanding of what actually happens in the field and in the packing house. The effect of different constantly maintained temperatures on the development of decay resulting from these inoculations with fungi singly and in various combinations of species was also investigated. Some previous investigation of the temperature relations of decay produced by *Penicillium italicum* and *P. digitatum* inoculated separately were made by Fawcett and Barger (4).⁴

It has been a common observation that two or more organisms may be associated in the same lesions and that in many cases the diseases appear to be made more severe by such association. Previous experiments with citrus fungi by Fawcett (2, 5) showed that certain mixed inoculations into citrus bark caused much more severe injury than any inoculation with a single fungus of the same group. For example, the injury caused by a mixed inoculation of *Diplodia natalensis* and *Colletotrichum gloeosporioides* was much greater than that caused by inoculations with either fungus alone. Inoculations with *Phytophthora citrophthora* combined with *Fusarium* sp. in California showed that the lesions of *Pythiacystis gummosis* progressed faster than when *P. citrophthora* was inoculated alone (3). On the other hand, one organism may hinder the attack or invasion of the other. For example, mixed inoculation by Fawcett (unpublished

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² Fellow, International Educational Board, 1926 and 1927.

³ Assistance in carrying on the technic of the experiments was given by Louis L. Huillier.

⁴ Reference is made by number (italic) to "Literature cited," p. 198.

data) of *Dothiorella gregaria* and *Pseudomonas juglandis* failed to cause appreciable injury, while a pure culture of *D. gregaria* produced large lesions when inoculated under the same conditions.

MATERIAL AND METHODS

The following species of fungi were isolated just previous to their use in the investigation, from fresh material, by the senior author; the numbers added are the stock culture numbers of the junior author at the Citrus Experiment Station, Riverside, Calif.: *Penicillium italicum* (1437) from lemon; *P. digitatum* (1438) from lemon; *Botrytis cinerea* (1439) from lemon; *Aspergillus niger* (1440) from orange; and *Sclerotinia libertiana* (1441) from lemon. The remainder listed below were cultures which had been isolated some months previous to their use, but were inoculated into fruits and reisolated in order to renew their vigor for the tests: *Oospora citri-aurantii* (1342) from lemon; *Pythiacystis citrophthora* (1309-A) from lemon bark; *Trichoderma lignorum* (1227-L) from lemon; *Alternaria citri* (1372-F) from orange; *Phomopsis californica* (1227-O) from lemon; *Diplodia natalensis* (1241-B) from grapefruit and *Dothiorella ribis* (1232-G) from lemon. All of these had been isolated from California fruits except *D. natalensis*, which came from a grapefruit grown in the Isle of Pines, Cuba. Glucose-potato agar was used for growing all cultures employed in the inoculation tests.

Several different preliminary experiments were made for the purpose of establishing the most satisfactory method of obtaining on the fruit a wound of uniform diameter and depth. At first a small-sized cork borer 4 millimeters in diameter, with a glass tube so placed over it as to limit the penetration to a certain determined point in the fruit, was used. Later, in order to facilitate sterilization, an all-metal instrument of the same size and diameter was made by attaching a metal shoulder around the outside of the cork borer instead of the glass tube. A metal wire was placed inside of the cork borer for the purpose of eliminating the disk of rind after each wound was made. With this instrument one could make uniform wounds in the surface of as many as 600 fruits in an hour. During the experiment the cork borer was sterilized by dipping it in 95 per cent alcohol and flaming after use on each group of 5 to 10 fruits. Before wounding, the fruit was washed and then disinfected with 95 per cent alcohol, which was rubbed over the surface by means of cotton and allowed to evaporate. The use of mercuric chloride was avoided because small amounts left on the rind might check the growth of the inoculum. The disinfection by alcohol proved quite satisfactory and allowed very little contamination, as shown by the almost complete absence of decay in the checks.

For the inoculation of the fruit, glass rods of uniform size with round, sanded ends were used. Frequently during inoculations the rod was dipped in alcohol and cooled before it was dipped into the suspension of spores.

Since it was difficult to avoid the possibility of some variation in the number of spores per inoculum, a preliminary investigation was made to find out what influence certain variations might have on the rate of decay. Six different series of inoculations with *Penicillium italicum* were tried, 20 oranges being used in each series. A spore

suspension containing approximately 2,000,000 spores per cubic centimeter of *P. italicum* was obtained by adding 10 cubic centimeters of sterile water to a 48-hour culture on a glucose-potato agar slant. The dilutions used were 1:1, 1:4, 1:5, 1:8, and 1:16 with sterile water. The average measurements taken every 48 hours (fig. 1)

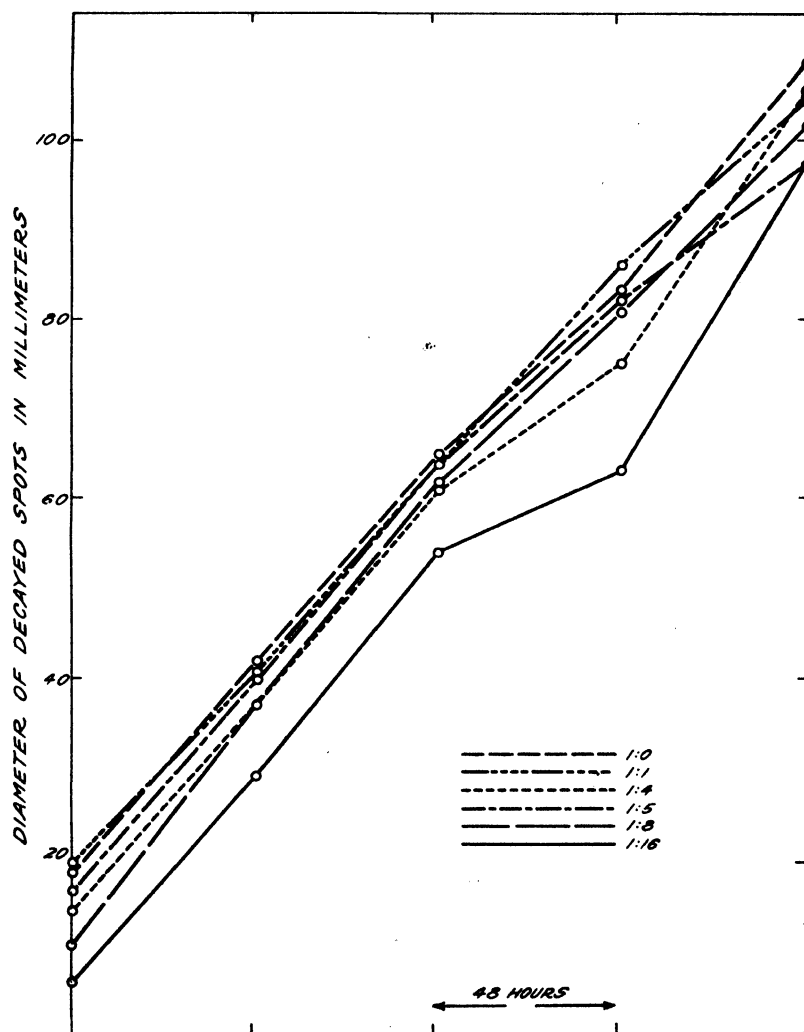


FIGURE 1.—Rates of decay of oranges after inoculations with different dilutions of a spore suspension of *Penicillium italicum*. The standard suspension contained about 2,000,000 spores per cubic centimeter

show that only when the inoculum was diluted sixteen times the volume of the original suspension was there an appreciable decrease in the rate of decay. In all other cases, as Figure 1 indicates, the slight differences in initiation of decay are related to the extent of dilution, but after four days there appears to be no definite rela-

tion between rate of decay and dilution. These suspensions represented a variation from about 2,000,000 to 125,000 spores per cubic centimeter. The experiment seems to indicate that within certain limits the number of spores in the inoculum has no deciding influence on the rate of decay. The density of spores was sufficient to start decay in all the fruits, so that the question of initiation of decay need not be considered.

In all the subsequent inoculations the density of the suspension of spores was so great that any ordinary variation in the inocula was probably not sufficient to make any difference in the rate at which decay proceeded. In mixed inoculations equal amounts of the original spore suspension of each fungus were shaken together. It was therefore concluded that the methods of inoculation used were probably sufficiently uniform to obviate large variations from this source.

The temperature apparatus used during the experiment was an improved type of that described by Livingston and Fawcett (?). The improvements consisted in larger temperature chambers with more nearly perfect insulation and the substitution of an electric refrigerator as a cooling unit in place of an ice compartment at one end, and of driving rods with beveled gears in place of wheels and belts underneath for the stirring apparatus. The entire apparatus was located in a basement room where temperature fluctuations throughout any 24 hours were small.

The temperature conditions were surveyed every time the inoculated fruit was to be taken out for observation, generally every 48 hours. Only the average temperature for the duration of each experiment is given. The variation during most of the experiments was from a few tenths to a half degree centigrade. The maximum variation during a few experiments when the apparatus was not in perfect running condition was about 1.5° C. The humidity of the chambers was also recorded in each experiment after the fruit was taken out.

To ascertain to what extent the humidity fluctuated during the complete process of rotting, a series of observations were taken every two days. (Table 1.)

TABLE 1.—*Relative humidity of different temperature chambers used in experiments on citrus-fruit decay*

Temperature of chamber	Relative humidity of chamber—	
	Four days after fruit was inoculated	Six days after fruit was inoculated
° C.	Per cent	Per cent
6.5	60	64
13.0	74	75
18.0	75	75
22.5	76	78
27.5	71	81
30.5	75	80
32.5	64	55

The variation in humidity in any of the chambers was probably not so great as to be an important factor in influencing the rate of decay. Since the fruit was wrapped and placed in paper sacks, the

humidity conditions surrounding it probably approached those surrounding packed fruit in cars on the way to market. In no case was the humidity so low as to allow noticeable wilting or excessive drying-out of the fruit during the period of the experiment.

EFFECT OF WATER LOSS ON RATE OF DECAY

In seasons when brown rot is prevalent or the fruit is unusually dirty and hot water is used for washing, it has been considered a good packing-house practice to hold fruit two or three days after picking before putting it into the hot water. This is known to prevent it from being injured by the hot water. The fruit thus held loses part of its turgidity, mostly because of water loss. The question arose as to what influence this might have upon the rate of decay. Apart from the question of external resistance to penetration of the fungus, would this loss of water give to the fruit some internal resistance to the progress of the fungus? An experiment designed to

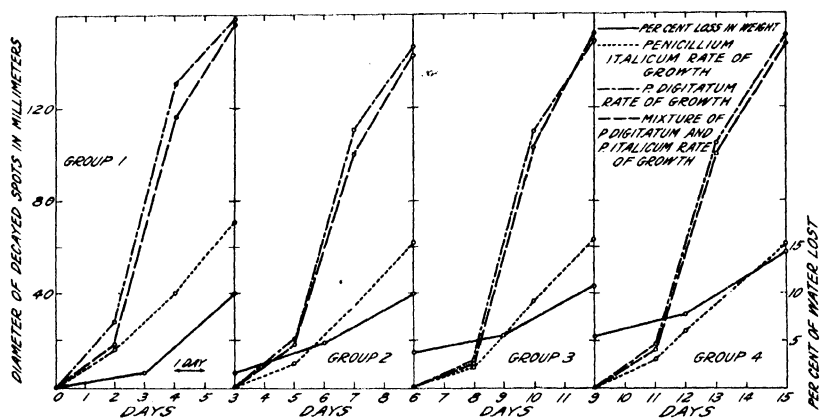


FIGURE 2.—Rate of decay and water loss of lemons inoculated with *Penicillium italicum* and *P. digitatum*, in relation to the period elapsing between the time of picking and of inoculation: Group 1 inoculated soon after picking; Group 2 on the third day; Group 3 on the sixth day; and Group 4 on the ninth day after picking

throw light on this point was performed as follows: A quantity of lemon fruits soon after picking was divided into four groups. The fruits of the first group were inoculated at once, those of the second in 3 days, those of the third in 6 days, and those of the fourth in 9 days. In each group there were 3 lots, 1 of 15 fruits inoculated with *Penicillium italicum*, 1 of 15 fruits inoculated with *P. digitatum*, and 1 of 20 fruits inoculated with a mixed spore suspension of the two fungi. These fruits were kept in a basement room at about 72° F. (22.2° C.) and about 43 per cent relative humidity during the experiment. After inoculation, the fruits of each group were placed in paper sacks. A careful record was taken every three days of the loss in weight of each group from picking until complete rotting of the fruit. This loss of weight is considered as loss of water. A record of the progress of rotting for each group was taken every 48 hours. The graphs shown in Figure 2 indicate that, with the exception of the group inoculated soon after picking, the rate of decay was almost the same whether the fruits were inoculated on

the third, sixth, or ninth day. The group inoculated soon after picking showed a rate of decay somewhat higher than the others. The total loss of water from picking to inoculation and during the period of rotting of the different lots is given in Table 2.

TABLE 2.—*Loss in weight (water loss) of groups of 50 lemons each, the rate of decay of which is shown in Figure 2*

[The fruits of Group 1 were inoculated immediately after picking; those of Groups 2, 3, and 4 were inoculated on the third, sixth, and ninth days, respectively, after picking]

Number of days after picking	Percentage loss in weight of lemons in—			
	Group 1	Group 2	Group 3	Group 4
0.....				
3.....	1.5	1.5	1.8	1.7
6.....	10.0	4.7	3.6	3.6
9.....		10.0	6.5	5.4
12.....			10.8	7.8
15.....				14.3

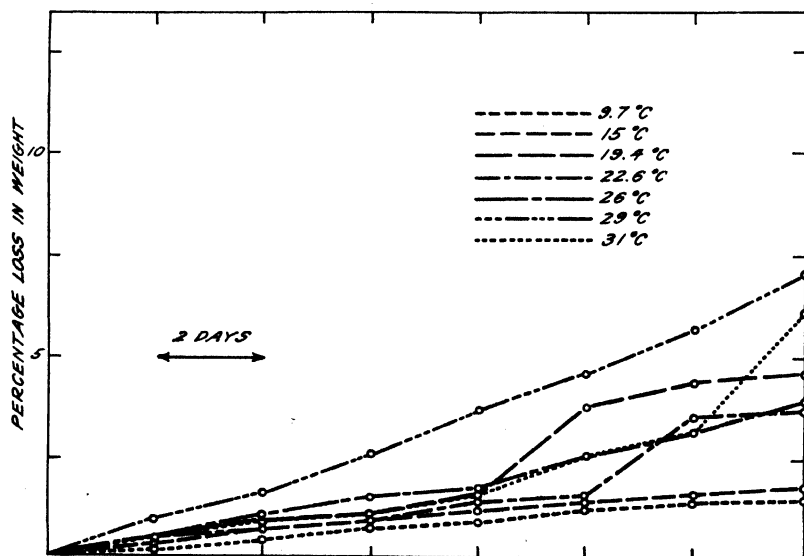


FIGURE 3.—Percentage of loss in weight on bases of initial weight in seven lots of yellow lemons held 14 days at 87 per cent humidity, and at varying temperatures, preceding the inoculations. (See also fig. 4)

EFFECT OF PREVIOUS CONSTANT TEMPERATURES ON RATE OF DECAY

To obtain some idea of the effect of the previous temperature conditions on subsequent rate of decay the following experiment was performed. Yellow lemon fruits directly from the field were divided into seven groups of 50 lemons each, uniform in size and color. These groups were weighed separately and put into different chambers of the temperature machine. Every two days a careful record was taken of the loss in weight of each group, constantly kept at the same temperature.

From the graphs in Figure 3 it is seen that the loss in weight of this fruit before inoculation increased with the temperature except at the highest one, 31° C.

After the fourteenth day the fruit was taken from the different chambers and left for 24 hours in the basement until it reached the temperature of the room, 22.4° C. (72.3° F.). The fruits were then inoculated with *Penicillium italicum*, with *P. digitatum*, and with a mixture of the two, and all were left in the basement at an air temperature averaging 22.4 and a humidity outside the bags of 42 per cent. The increment of rotting was measured every day for nine days, starting from the third day.

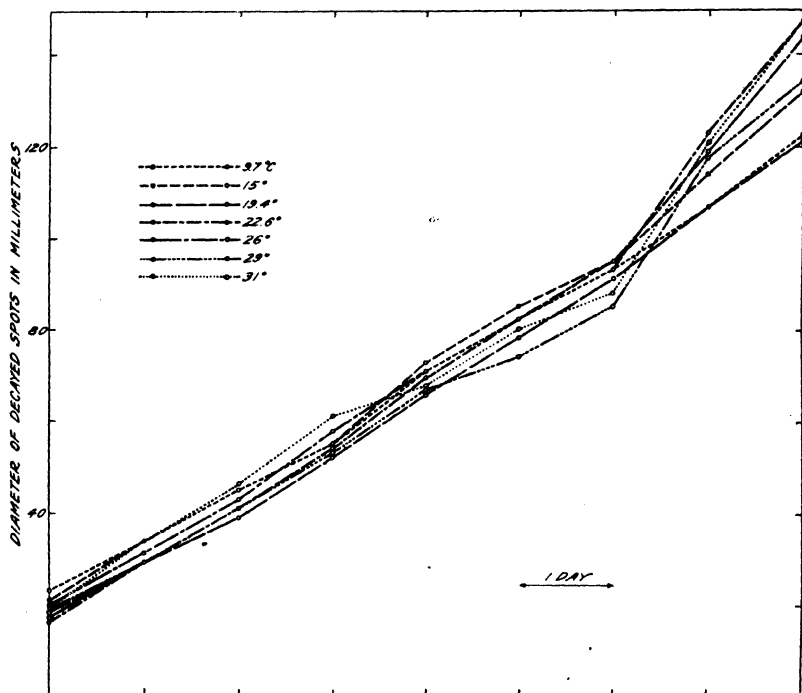


FIGURE 4.—Rate of decay of seven lots of yellow lemons (tree-ripe fruit) inoculated with *Penicillium italicum* and held at 22.4° C. Just prior to inoculation the lots had been subjected to 87 per cent humidity, at the temperatures indicated, for a period of 14 days

The graph for *Penicillium italicum* (fig. 4), which is representative of the three lots, seems to show that there is no definite relation between the previous exposure to different temperatures for an initial period of two weeks from picking and the subsequent rate of decay from uniform inoculation.

EFFECT OF ALTERNATING TEMPERATURES ON RATE OF DECAY

The question arose during the investigation as to the possible effect on decay of alternating temperatures as compared to constant temperatures.

Experiments by McKinney (8) had shown that for *Helminthosporium sativum* the effect of an alternating temperature, low during

the night and higher during the day, was practically equal to a constantly maintained temperature that was near the average of the two extremes. To make a comparison of this kind between constant and alternating temperatures, the following experiment was performed. The fruit from the field was left in the basement for 24 hours to acquire the room temperature, about 21° C. The next day two groups of lemons, each of three lots, were inoculated with *Penicillium italicum* alone, *P. digitatum* alone, and a combination of the two. The fruit was wrapped in regular citrus paper, each group put in a separate paper bag and then placed in chambers, one at 9.8° and the other at 27.1°. One group was left undisturbed in the two chambers as a check; the other was alternated every 12 hours from the highest to the lowest temperature chamber, and vice versa. Another check was left in a chamber at 20°, which temperature was nearest to the average of the two extremes. Measurements of the increment of rotting of the fruit were taken every 12 hours, except the first measurement, which was taken after 24 hours. The lots of fruit inoculated respectively with *P. italicum* and *P. digitatum* which

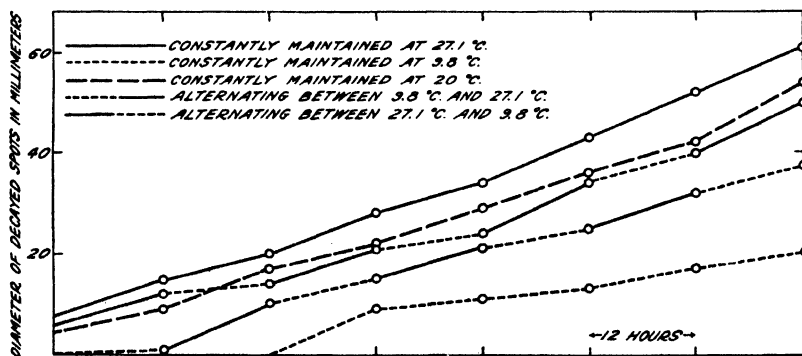


FIGURE 5.—Rate of decay of lemons inoculated with *Penicillium italicum* and stored at alternating temperatures as compared with rate of decay when stored at constant temperatures

had been alternated between 9.8° and 27.1° C. after an initial exposure of 24 hours to the higher temperature of 27.1°, showed average rates of decay not very far from those of the fruits that had remained in a chamber at 20° and were not alternated. On the other hand, the lots which were alternated between the same 9.8° to 27.1° after an initial exposure to the lower temperature, 9.8°, showed rates of decay below those held at a constant temperature of 20°. This is illustrated in Figure 5 for *P. italicum*.

The slope of the graphs for rate of decay of the fruits in alternated temperatures change with each change of temperature and become almost parallel with the slopes of the corresponding graphs at the same temperature as the fruits not alternated.

LAG IN FRUIT TEMPERATURES AFTER INOCULATION

The time required for the temperature of oranges and lemons to come to the temperature of the chambers was tested to determine the effect of the lag in temperature on initial decay. The first test was made with average-sized lemons about 150 millimeters in circum-

ference, unwrapped. The fruit, carefully selected for size and color, was divided into two lots. In one lot thermometers were inserted in the center of the fruit and in the other they were inserted under the rind. The thermometers were sealed in the fruit with paraffin to prevent air from entering along the stem. The fruit was left in the extreme chambers, 49° F. (8.3° C.) and 88° F. (31.1° C.) overnight until the two lots were found to have attained the temperature of the respective chambers. The two lots were then interchanged—the lot from the higher temperature chamber was removed to the lower temperature chamber, and that from the lower temperature chamber to the higher.

The temperature just under the rind changed somewhat more rapidly at first than that in the center of the fruit, but after three to four hours both were the same, and in five hours the two lots had attained the temperature of the chamber in which they were placed.

In another experiment the fruit was wrapped and placed in paper sacks, as was done in most of the subsequent inoculation experiments. In one group the thermometers were inserted through the wrapper just inside the rind and in the other group they were inserted in the center of the fruits. The initial temperature of all the fruit was about 26.5° C. Different lots were placed in chambers at temperatures of 9°, 20.5°, 28°, and 34.5° C., and the temperature at intervals of two hours was recorded. Table 3 shows the results of this test. Differences of temperature of from 6.5° to 8° C. between the interior of the wrapped and bagged fruit was overcome completely in 6 to 10 hours, and a difference of 17.5° was overcome in 12 hours.

TABLE 3.—Change in temperature of wrapped lemons exposed in chambers at four different air temperatures

[Initial fruit temperature, 26.5° C.]

Temperature of chamber (° C.)	Position of thermometer in fruit	Temperature of fruit after exposure for—					
		2 hours	4 hours	6 hours	8 hours	10 hours	12 hours
		° C.	° C.	° C.	° C.	° C.	° C.
9.0.....	(At center.....	18.0	14.5	12.0	11.0	10.0	9.0
	(Just under rind.....	17.0	14.0	12.0	11.0	10.0	9.0
20.5.....	(At center.....	23.5	22.5	20.5	20.5	20.5	20.5
	(Just under rind.....	22.5	21.5	20.5	20.5	20.5	20.5
28.0.....	(At center.....	26.5	27.0	27.5	27.5	28.0	28.0
	(Just under rind.....	26.5	27.0	27.5	27.5	28.0	28.0
34.5.....	(At center.....	30.0	32.0	33.0	34.0	34.5	34.5
	(Just under rind.....	32.0	33.0	34.0	34.5	34.5	34.5

COLOR, RESISTANCE TO PUNCTURE, AND RATE OF DECAY

Observation had indicated that green lemon fruits decay less rapidly than yellow ones. From about 600 lemons of the same size, eight groups were selected showing gradations of color from deep green to deep yellow. (Table 4.)

TABLE 4.—Approximate color ^a of eight groups of lemons before and after inoculation with *Penicillium italicum*

Group No.	Color before inoculation	Color after the last observation
1.....	Green yellow.....	Mustard yellow.
2.....	Greenish yellow.....	Strontian yellow.
3.....	Lemon yellow.....	Do.
4.....	Lemon chrome.....	Aniline yellow.
5.....	Cress green.....	Amber yellow.
6.....	Light cress yellow.....	Do.
7.....	Absinthe green.....	Primuline yellow.
8.....	Citron green.....	Lemon chrome.

^a According to Ridgway's Color Standards (9).

After the resistance to puncture was measured with a penetrometer (6) each fruit was inoculated with *Penicillium italicum*.⁵ The fruit

was then left at a temperature of 70° to 75° F., and the rate of growth of the fungus was recorded every 48 hours. The graphs representing the averages of each group (fig. 6) show a close relation between rate of decay and resistance to penetration and also between rate of decay and color.

The relationship between resistance to penetration and rate of decay is shown by the graphs in Figure 7 when the individual fruits were divided into groups differing by 50 grams of pressure without reference to color. Here the average rate of decay of the different groups is distinctly decreased

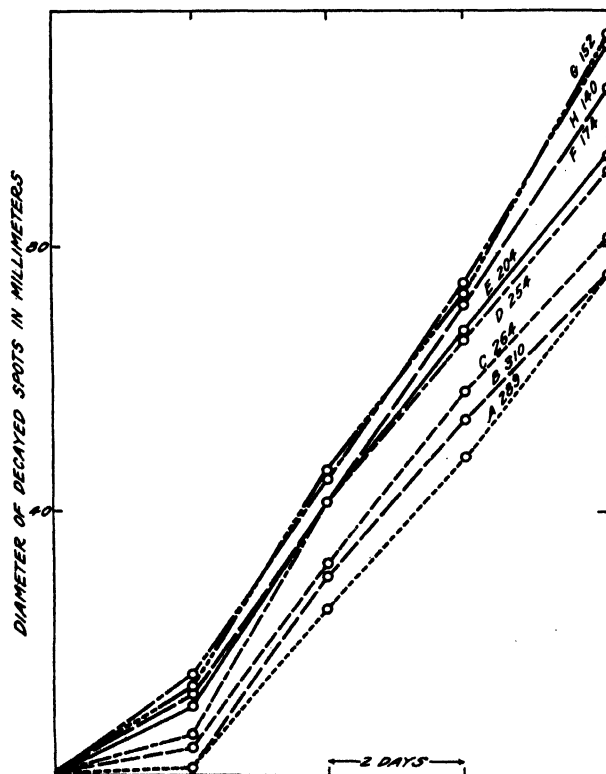


FIGURE 6.—Rate of decay of lemons as related to their color from dark green (A) to light yellow (H), and to resistance to penetration. The resistance to pressure as measured with a penetrometer and expressed in grams averaged as follows for lemons in each group: A, 289; B, 310; C, 264; D, 254; E, 204; F, 174; G, 152; H, 140

as the resistance to pressure increases. The experiment indicated that color was a fairly good criterion for the selection of fruit with approximate uniformity of resistance to pressure and also uniformity of

⁵ These measurements were made with the help of E. R. Parker, of the Citrus Experiment Station.

resistance to rate of decay by *P. italicum*. Fruits for the different sets of subsequent experiments were selected for approximate uniformity of size and color in order to avoid undue individual variability.

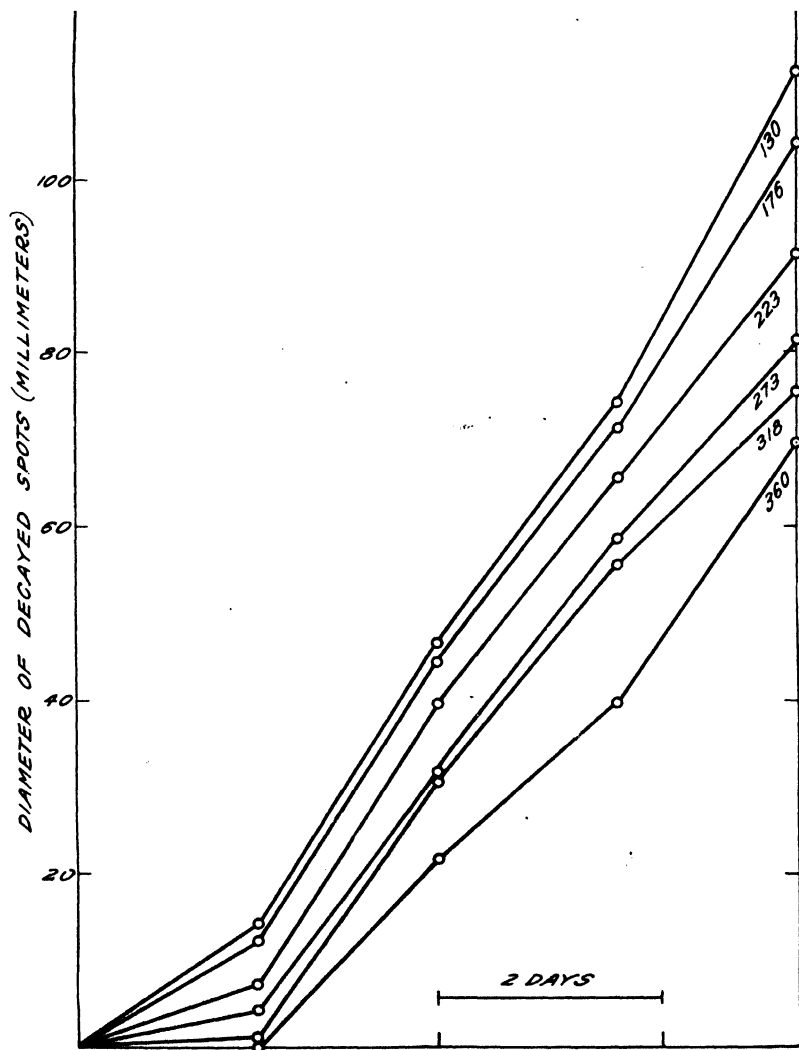


FIGURE 7.—Rate of decay of lemons as related to pressure (expressed in grams) necessary to puncture the rind

COMBINED INOCULATIONS OF *PENICILLIUM ITALICUM* AND *P. DIGITATUM*

Oranges and lemons were inoculated as previously described with a mixed suspension of spores of *Penicillium italicum* and *P. digitatum*. For comparison a second group of fruits was inoculated with *P. italicum* alone, a third with *P. digitatum* alone, and a fourth was injured only as a check. The first group contained 70 lemons and 70

oranges divided into seven equal lots of 10 lemons and 10 oranges, and a lot was placed at each of the seven temperatures shown in Table 5. The second, third, and fourth groups contained 35 lemons and 35 oranges divided into lots of 5 lemons and 5 oranges and distributed like the first group.

The measurements in millimeters were made from margin to margin of the decayed spots through the place of inoculation. The fraction of a millimeter resulting from the computation of the averages was not considered if it was less than one-half millimeter, if more it was considered as unity.

During the experiment several points were taken into consideration, namely, sporulation of the fungi inside and outside the fruit, appearance of mycelium on the surface, discolorations during the process of rotting inside and outside of the fruit, general differences between the series of combined inoculated fruit and the other series inoculated with single organisms; and isolation of the fungi in the combined inoculation series from day to day to determine whether or not the rotting tissues inside the fruit contained the same fungus as that shown on the outside.

APPEARANCE OF MYCELIUM ON THE SURFACE OF THE FRUIT AND SPORULATION

In both lemons and oranges *Penicillium italicum* sporulated at a lower temperature than *P. digitatum*. The sporulation range of the former was from 9.8° to 29.8° C. (most abundant spores at about 20°) and of the latter from 17.8° to 29.8° (most abundant spores at about 27.6°) in eight days.

In the combined inoculations of lemon, the mycelium of both fungi was observed on the same fruit. The blue spores of *Penicillium italicum* appeared at a lower temperature than the green spores of *P. digitatum*, which occurred at no temperature below 17.8° C. in the eight days of the experiment. Above this temperature the sporulation of *P. italicum* was confined to a few millimeters around the inoculation wound, while sporulation of *P. digitatum* became abundant in a few days over the remainder of the decayed areas. In the cases studied the area of mycelium of *P. digitatum* as compared to the size of the spot diminished as the distance from the optimum temperature (about 25°) for rate of decay increased. In the combined inoculation, sporulation of *P. italicum* was slightly inhibited by the presence of *P. digitatum*. As decay due to *P. italicum* advanced, sporulation inside at the core was noted. No sporulation of *P. digitatum* inside the fruit was observed. At all temperatures used the rot started in the rind and advanced into the pulp.

DISCOLORATIONS DURING ROTTING AND DIFFERENCES IN COMBINED AND SINGLE INOCULATIONS

Both in oranges and in lemons in all the series inoculated, whether with a mixed or a single inoculum, there appeared at temperatures above 21° C. a brown marginal discoloration 5 to 15 millimeters in width at the extreme advancing margin of decay. At the highest temperature the color was darker than at other temperatures; the lemons were clove brown and the oranges were sayal brown (Ridgway (9)). The discoloration seemed to have been produced by the higher temperatures.

The combination of the two species of *Penicillium* produces a characteristic reddish or pinkish discoloration on and in the rind

around the inoculation point for 5 to 10 millimeters. This kind of discoloration has never been found in inoculations of *Penicillium italicum* or *P. digitatum* alone. When such fruits are cut through the inoculated wound there is found a reddish discoloration of the tissue underlying the rind. (Fig. 8.) This discoloration is an aid in identifying mixed infections of these fungi, as has been pointed out by Fawcett and Lee (5).

As is shown in Table 5, in which are compared the rates of growth on the fourth and sixth days, the combined inoculation in lemons produced an increased rate of decay (fig. 9) at the low temperatures

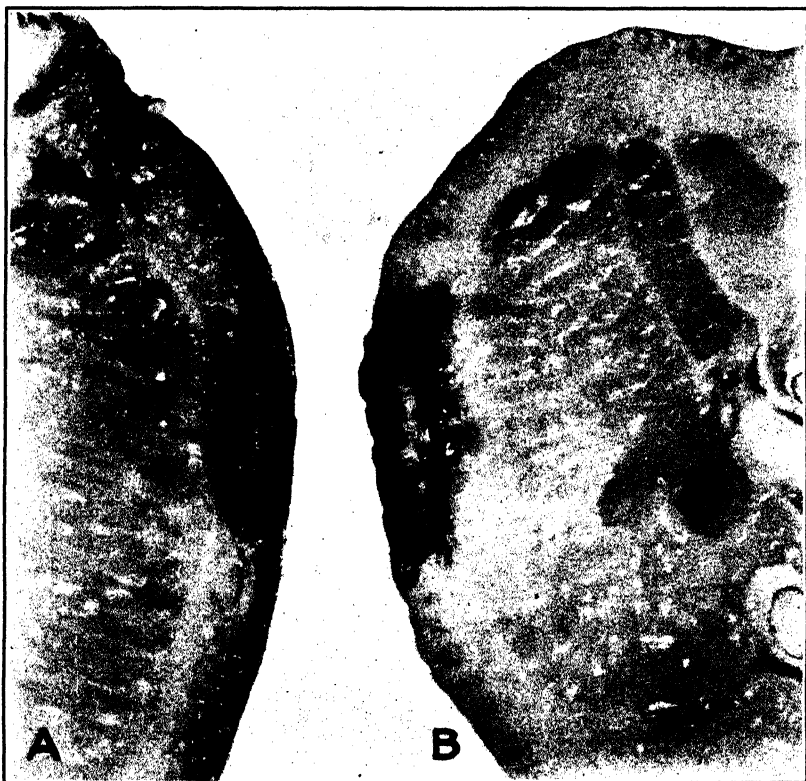


FIGURE 8.—Decay in inoculated fruit; the dark portion indicates the red discoloration typical of decay after a mixed inoculation with *Penicillium italicum* and *P. digitatum*: A, In lemon; B, in orange

9.8° and 14.1° C. and at the high temperatures 27.6 and 29.8°, as compared with *Penicillium digitatum*, but slight differences at intermediate temperatures. In oranges there is an indication that the rate of growth of the mixture is the same or slightly lower than that of *P. digitatum* alone. To determine whether or not the same fungus was present in the interior of the fruit as appeared from the sporulation on the surface, a series of daily isolations were made as shown in Table 6. In cases where more than one organism occurred in the cultures the first mentioned was the most prominent. In making such cultural tests the following method was used. Ten lemons and

10 oranges were inoculated on the same day with *Penicillium italicum* and *P. digitatum* and kept at 21.5° C. (71° F.). Every 24 hours two oranges and two lemons were taken for isolations. Before these were cut they were washed for 10 minutes in tap water to carry away

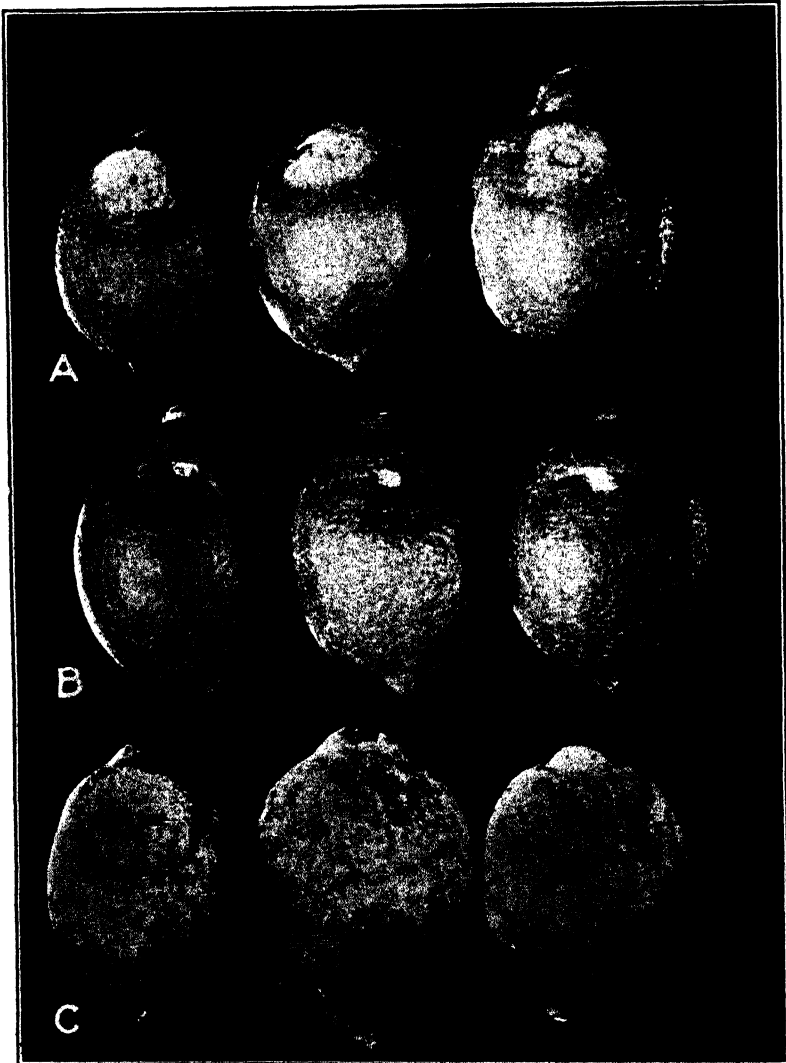


FIGURE 9.—Increased rate of decay in lemons produced by a mixed inoculation with *Penicillium italicum* and *P. digitatum* (C) as compared with inoculations of *P. digitatum* alone (A), or *P. italicum* alone (B); fruit held at 9.8° C. for eight days

a large number of the spores and at the same time to give to the remainder a sticky consistency and so prevent them from flying about during the isolation. In order to avoid contaminations three sterile scalpels were used in cutting, one for the advanced margin, another

for the place midway from the margin to the inoculation, and the last for the region under the inoculation. A small piece of decayed material in each test was transferred to glucose-potato agar.

TABLE 5.—Rate of decay at different temperatures of lemons and oranges inoculated with two species of fungi separately and in combination

LEMONS								
Inoculum	Days after inoculation	Diameter in millimeters of decayed spots on fruit kept at—						
		9.8° C.	14.1° C.	17.8° C.	21.2° C.	25.2° C.	27.6° C.	29.8° C.
<i>Penicillium italicum</i>	4	0	12	26	35	31	21	9
	6	10	26	48	62	61	37	10
<i>P. digitatum</i>	4	0	0	45	79	88	44	11
	6	11	33	120	* 150	* 150	* 150	12
<i>P. italicum</i> + <i>P. digitatum</i>	4	0	19	37	82	93	72	18
	6	22	48	86	* 150	* 150	* 150	24
ORANGES								
<i>P. italicum</i>	4	0	12	26	34	40	35	40
	6	12	25	47	60	66	57	90
<i>P. digitatum</i>	4	0	22	48	73	92	95	77
	6	20	60	104	* 200	* 200	* 200	101
<i>P. italicum</i> + <i>P. digitatum</i>	4	0	20	46	73	86	93	76
	6	15	55	106	* 200	* 200	* 200	93

* Entire circumference of fruit.

TABLE 6.—Results of daily culture tests from decayed spots on fruits previously inoculated with a mixed spore suspension of *Penicillium italicum* and *P. digitatum* and kept at 21.5° C.

LEMONS			
Number of days after inoculation at which isolation tests were made	Place in the decayed spot from which culture tests were made		
	Advancing margin of decay	Midway from margin to wound	Under the inoculated wound
1.....	No growth	No growth	No growth.
2.....	<i>P. digitatum</i>	<i>P. italicum</i> + <i>P. digitatum</i>	<i>P. italicum</i> + <i>P. digitatum</i> .
	do	do	Do.
3.....	do	<i>P. digitatum</i>	Do.
	do	do	Do.
4.....	do	do	Do.
	do	<i>P. italicum</i> + <i>P. digitatum</i>	Do.
5.....	do	do	<i>P. italicum</i> .
	do	do	Do.
ORANGES			
1.....	No growth	No growth	No growth.
2.....	<i>P. digitatum</i>	<i>P. italicum</i> + <i>P. digitatum</i>	<i>P. italicum</i> + <i>P. digitatum</i> .
	do	do	Do.
3.....	do	<i>P. digitatum</i>	Do.
	do	do	Do.
4.....	do	<i>P. digitatum</i> + <i>P. italicum</i>	Do.
	do	<i>P. italicum</i> + <i>P. digitatum</i>	Do.
5.....	do	<i>P. digitatum</i>	<i>P. italicum</i> .
	do	do	Do.

The results in Table 6 indicate that underneath the surface of the decay produced by a mixed inoculum of *Penicillium italicum* and *P. digitatum* at the favorable temperature of 21.5° C., *P. digitatum* will

be present at the advancing margins, either one or both will be present midway from the margin to the center, and both will live under the inoculated wound during the first few days, but after five days *P. digitatum* will be inhibited, leaving only *P. italicum* capable of being recovered.

COMBINED INOCULATIONS OF ASPERGILLUS NIGER, PENICILLIUM ITALICUM, AND P. DIGITATUM

TEMPERATURE RELATIONS OF ASPERGILLUS NIGER

This experiment was carried out by the methods previously described, at seven different constant temperatures with inoculations of five groups of lemons and five groups of oranges, as follows: (1) *Aspergillus niger*, (2) *A. niger* + *Penicillium italicum*, (3) *A. niger* + *P. digitatum*, (4) *A. niger* + *P. italicum* + *P. digitatum*, and (5) checks. The first and fifth groups contained each 35 lemons and 35 oranges divided into seven lots of 5 lemons and 5 oranges, and a lot was placed at each of the seven temperatures shown in Table 7. The second, third, and fourth groups contained 70 lemons and 70 oranges divided into lots of 10 lemons and 10 oranges and distributed like the first group.

During the eight days of the experiment no decay was evident from 9.6° to 19.3° C. either in lemons or oranges. After three days rotting had started at 23° and at 32.2°, developing rapidly at the latter temperature. Slight sporulation was nearly always contemporaneous with the beginning of decay. At high temperatures a pronounced white mycelial ring appeared in advance of the decay and in the center of this a black mass of spores was formed.

TEMPERATURE RELATIONS OF THE COMBINATIONS ASPERGILLUS NIGER + PENICILLIUM ITALICUM, A. NIGER + P. DIGITATUM, AND A. NIGER + P. ITALICUM + P. DIGITATUM

The rate of decay produced by the three combinations used in this group of experiments is shown in Table 7.

TABLE 7.—Rate of decay at different temperatures of lemons and oranges inoculated with *Aspergillus niger* alone and in combination with other fungi

LEMONS								
Inoculum	Days after inoculation	Diameter in millimeters of decayed spots on fruit kept at—						
		9.6° C.	14.7° C.	19.3° C.	23° C.	27.3° C.	30.1° C.	32.2° C.
<i>A. niger</i>	4	0	0	0	18	20	37	36
	6	0	0	0	32	42	59	60
<i>A. niger</i> + <i>P. italicum</i>	4	0	24	34	58	35	35	44
	6	16	42	60	66	50	53	63
<i>A. niger</i> + <i>P. digitatum</i>	4	0	44	78	113	103	36	49
	6	15	92	• 150	• 150	• 150	53	71
<i>A. niger</i> + <i>P. italicum</i> + <i>P. digitatum</i>	4	16	45	86	97	70	35	45
	6	30	93	• 150	• 150	• 150	54	57
ORANGES								
<i>A. niger</i>	4	0	0	0	17	27	42	41
	6	0	0	0	27	42	65	78
<i>A. niger</i> + <i>P. italicum</i>	4	0	18	30	38	29	42	49
	6	16	32	42	63	43	67	77
<i>A. niger</i> + <i>P. digitatum</i>	4	0	38	64	89	91	46	55
	6	28	80	111	-----	115	64	80
<i>A. niger</i> + <i>P. italicum</i> + <i>P. digitatum</i>	4	16	41	66	89	102	47	50
	6	46	80	• 200	• 200	• 200	62	78

• Entire circumference of fruit.

The mixtures *Aspergillus niger* + *Penicillium digitatum* and *A. niger* + *P. italicum* + *P. digitatum*, showed higher rates of decay than the highest rate of any one of the fungi alone for both oranges and lemons at the two lowest temperatures, 9.6° and 14.7° C. (Compare Tables 5 and 7.) In lemons this increased rate extended up to 27.3°. A mixture of *A. niger* with *P. italicum* produced an increased rate at the lowest temperature, 9.6° in lemons and at both 9.6° and 14.7° in oranges. At higher temperatures than those mentioned the rates of the mixtures were equal to or slightly less than the highest rate of any component under the same conditions. The external characters of the combination *A. niger* + *P. italicum* were the same as those of pure *P. italicum* from 9.6° to 19.3°. *A. niger* occurred in the center for 5 to 20 mm., followed by *P. italicum*, which extended to the advancing margin at 23° to 27.3°. Only *Aspergillus* was found at 30° and above. No striking differences in the external appearance of decay on oranges and lemons were noted.

The external characters of the combination *Aspergillus niger* + *P. digitatum* were those of pure *P. digitatum* from 9.6° to 23° C., above which the *A. niger* appeared in the center over an area 5 to 22 mm. in diameter until a temperature of 30.1° and 32.2° was reached, when *A. niger* only was evident.

The external characters of the combination *Aspergillus niger* + *Penicillium italicum* + *P. digitatum* were similar to those of the combination *P. italicum* + *P. digitatum* already described at temperatures of 9.6 to 23° C. At 27.3° *A. niger* appeared in the center of the decayed area, followed concentrically by *P. italicum* and then by *P. digitatum* up to the advancing margin. At 31.2° only *A. niger* was evident.

CULTURAL TESTS FROM TISSUES INSIDE THE FRUIT

At the end of the experiment fruits inoculated with the combination *Aspergillus niger* + *Penicillium italicum* + *P. digitatum* from each of the seven chambers were used in isolation tests in order to find out what changes were taking place within the fruit in relation to those that showed on the surface. These tests were made from the "albedo" or inner part of the rind in the manner previously described in connection with *P. italicum* + *P. digitatum*. The results are shown in Table 8.

TABLE 8.—Isolation culture tests from decayed spots on fruits inoculated with *Aspergillus niger* + *Penicillium italicum* + *P. digitatum* eight days previously

LEMONS

Temperature (° C.)	Place in decayed spots from which culture tests were made		
	Advancing margin	Midway from margin to inoculation	Under the inoculated wound
9.6.....	<i>P. italicum</i>	<i>P. italicum</i>	<i>P. italicum</i> + <i>A. niger</i> .
14.7.....	<i>P. digitatum</i>	<i>P. italicum</i> + <i>P. digitatum</i>	Do.
19.3.....	do.....	do.....	<i>P. italicum</i> .
23.....	do.....	do.....	<i>A. niger</i> .
27.3.....	do.....	<i>P. digitatum</i> + <i>P. italicum</i>	Do.
30.1.....	<i>A. niger</i> + <i>P. digitatum</i>	<i>A. niger</i> + <i>P. digitatum</i> + <i>P. italicum</i>	Do.
32.2.....	<i>A. niger</i>	<i>A. niger</i>	<i>A. niger</i> + <i>P. italicum</i> .

TABLE 8.—Isolation culture tests from decayed spots on fruits inoculated with *Aspergillus niger* + *Penicillium italicum* + *P. digitatum* eight days previously.—Con.

ORANGES

Temperature (° C.)	Place in decayed spots from which culture tests were made		
	Advancing margin	Midway from margin to inoculation	Under the inoculated wound
9.6.....	<i>P. italicum</i> + <i>P. digitatum</i> .	<i>P. italicum</i> + <i>P. digitatum</i>	<i>P. italicum</i> + <i>A. niger</i> .
14.7.....	<i>P. digitatum</i>	do.....	<i>P. italicum</i> + <i>P. digitatum</i> .
19.3.....	do.....	<i>P. digitatum</i> + <i>P. italicum</i>	Do.
23.....	do.....	<i>P. digitatum</i>	Do.
27.3.....	do.....	<i>P. digitatum</i> + <i>P. italicum</i>	<i>A. niger</i> .
30.1.....	<i>A. niger</i> + <i>P. digitatum</i> .	<i>A. niger</i> + <i>P. italicum</i> + <i>P. digitatum</i> .	<i>A. niger</i> + <i>P. italicum</i> .
32.2.....	<i>A. niger</i>	<i>A. niger</i> + <i>P. italicum</i>	Do.

In the advancing margin at the lowest temperature, 9.6° C., *Penicillium italicum* was found associated with the rotting of the fruit; from 14.7° to 27.3°, *P. digitatum*; at 30.1°, *Aspergillus niger* + *P. digitatum*; and at 32.2, *A. niger* alone was found. Since the spores of the various organisms were present in approximately the same amounts at the beginning, it is apparent that different temperatures exerted a selective influence, allowing one fungus in the mixture to become dominant at certain temperatures and permitting others to become dominant at other temperatures. It should also be noted that the fungi found under the surface did not always coincide with that which was seen sporulating on the surface above the place of isolation. At the completion of the experiment the fruit was taken out of the chambers and the lots were arranged in the order of the magnitude of the temperatures at which they had been kept. The effect produced by the combination *A. niger* + *P. italicum* + *P. digitatum* was very striking. The fruits from the lower-temperature chambers were predominantly blue in color due to spores of *P. italicum*; those from slightly higher temperatures showed a reduction of the blue color in the center with the green of *P. digitatum* spores predominating; those from still higher temperatures showed the black of *A. niger* spores in the center with the green of *P. digitatum* around them, and those at the highest temperatures showed only the black of *A. niger* spores.

COMBINED INOCULATIONS OF OOSPORA CITRI-AURANTII, PENICILLIUM ITALICUM, P. DIGITATUM, AND ASPERGILLUS NIGER

TEMPERATURE RELATIONS OF OOSPORA CITRI-AURANTII

Oospora citri-aurantii has, in comparison with many other rot fungi, a very wide range of temperature for decay. In lemons, as will be shown in the low-temperature experiments, it had during the six days of the experiment a minimum of about 7° to 8° C., an optimum of about 27° to 28°, and a maximum above 33°; in oranges it had a minimum of about 5° to 6°, an optimum of about 29° to 30°, and a maximum above 33° for the same time. In general the rot is soft in the beginning, but later becomes sunken and watery. In lemons the decay is much softer and more watery than in oranges. When completely rotted, the fruit is collapsed. Sporulation started

on lemons eight days after inoculation at a temperature of 23.5° and on oranges eight days after at 19.8°, and was absent on both lemons and oranges at 30° or above during the same period.

TEMPERATURE RELATIONS OF *OOSPORA CITRI-AURANTII*, *PENICILLIUM ITALICUM*, *P. DIGITATUM*, AND *ASPERGILLUS NIGER*

An experiment was carried on at each of seven maintained temperatures with six lots of lemons and six lots of oranges as follows: (1) *Oospora citri-aurantii* alone, (2) *O. citri-aurantii* + *Penicillium italicum*, (3) *O. citri-aurantii* + *P. digitatum*, (4) *O. citri-aurantii* + *P. italicum* + *P. digitatum*, (5) *O. citri-aurantii* + *P. italicum* + *P. digitatum* + *Aspergillus niger*, (6) check. Each of these groups contained 35 lemons and 35 oranges divided into seven lots of 5 lemons and 5 oranges, and a lot was placed at each of the seven temperatures shown in Tables 9 and 10.

TABLE 9.—Rate of decay at different temperatures of lemons and oranges inoculated with *Oospora citri-aurantii* alone and in combination with other fungi

LEMONS								
Inoculum	Days after inoculation	Diameter in millimeters of decayed spots on fruit kept at—						
		11.5° C.	15.9° C.	19.8° C.	23.5° C.	27.2° C.	30.2° C.	31.9° C.
<i>O. citri-aurantii</i>	4	9	10	18	26	33	33	32
	6	16	20	43	49	59	57	46
<i>O. citri-aurantii</i> + <i>P. italicum</i>	4	14	15	30	40	38	39	42
	6	29	44	60	62	61	63	56
<i>O. citri-aurantii</i> + <i>P. digitatum</i>	4	15	29	67	108	107	64	42
	6	62	* 150	* 150	* 150	* 150	76	63
<i>O. citri-aurantii</i> + <i>P. italicum</i> + <i>P. digitatum</i>	4	17	48	97	118	107	101	43
	6	83	* 150	* 150	* 150	* 150	115	63
ORANGES								
<i>O. citri-aurantii</i>	4	0	0	11	12	21	29	29
	6	0	10	27	29	45	55	52
<i>O. citri-aurantii</i> + <i>P. italicum</i>	4	12	21	29	38	22	41	45
	6	24	44	61	64	65	67	67
<i>O. citri-aurantii</i> + <i>P. digitatum</i>	4	11	32	58	83	95	87	37
	6	42	75	116	* 200	* 200	121	57
<i>O. citri-aurantii</i> + <i>P. italicum</i> + <i>P. digitatum</i>	4	14	32	65	90	100	96	35
	6	45	80	118	* 200	* 200	117	55

* Entire circumference of fruit.

In lemons, as shown in Table 9, the combination *Oospora citri-aurantii* + *Penicillium italicum* produced a more rapid decay than *O. citri-aurantii* or *P. italicum* alone (compare with Table 5), and the combination *O. citri-aurantii* + *P. digitatum* produced a decidedly more rapid growth than did *P. digitatum* or *O. citri-aurantii* alone; in fact at lower and intermediate temperatures it was equal to or greater than the sum of the rates of growth of *O. citri-aurantii* and *P. digitatum* when used alone.

In oranges the rate of decay caused by *Oospora citri-aurantii* + *Penicillium digitatum* was slightly greater at 11.5° to 23.5° C. than that caused by *P. digitatum* alone, but above these temperatures the growth was about the same except at the highest temperature (31.9°), where it was less. The combination *O. citri-aurantii* + *P. digitatum*

in the first two chambers (11.5° and 15.9°) gave an increment of decay somewhat greater than the sum of the increments given by *O. citri-aurantii* and *P. digitatum* alone, but from 19.8° to 31.9° it gave an increment constantly somewhat less than the sum. On the surface of lemons inoculated with *O. citri-aurantii* + *P. italicum* the spores of *P. italicum* were limited to a few millimeters at a temperature of 11.5° , but at 23.5° this rot had reached a size of 25 mm.;

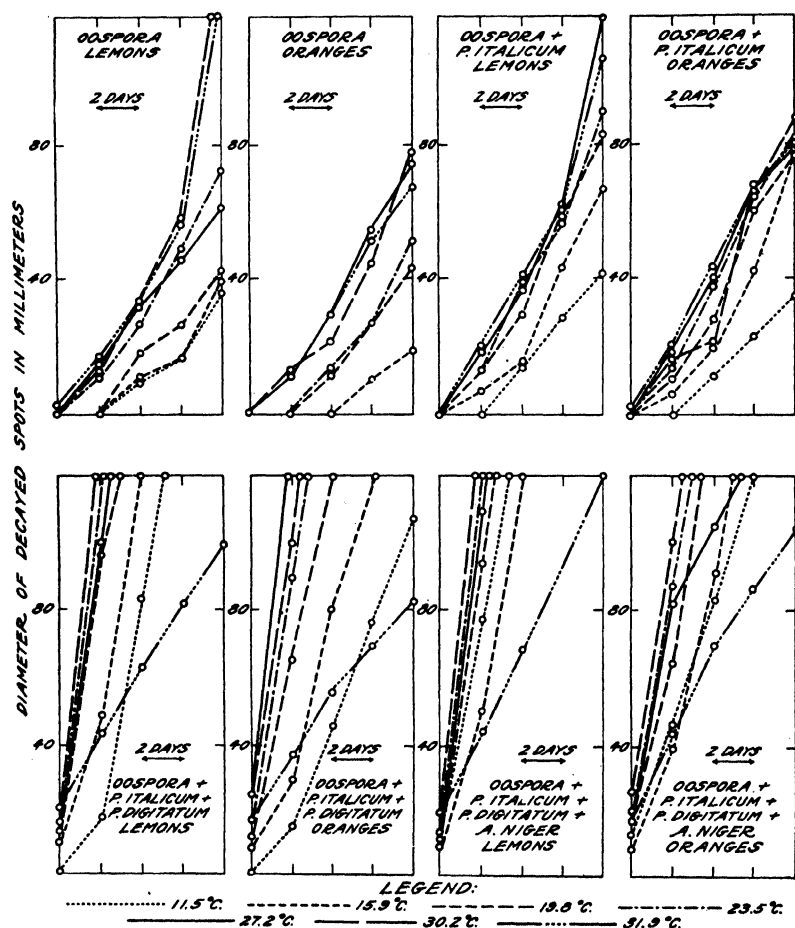


FIGURE 10.—Diameter of decayed areas on lemons and oranges produced by inoculations with *Oospora citri-aurantii* alone and mixed with other fungi; fruits were held at temperatures indicated for an 8-day period

above 23.5° only spores of *O. citri-aurantii* were seen. In all cases *O. citri-aurantii* was growing on the advancing margin of the decay. In oranges a similar manifestation was noted, with the difference that the spores of *P. italicum* were still present at 30° .

On the surface of the decayed areas resulting from inoculating oranges with *Oospora citri-aurantii* + *Penicillium digitatum*, only the mycelium of *P. digitatum* was evident at temperatures of 11.5° and

15.9° C. From 19.8° to 31.9° the spores of *O. citri-aurantii* were in the center of the area, and at 27.2° this rot had reached a diameter of 15 mm.

The combination in all cases resulted in *P. digitatum* actively growing in the advancing margin of the rot.

When lemons were inoculated with *Oospora citri-aurantii* + *Penicillium digitatum* oospora occurred at temperatures of 15.9° to 31.9° C. At 15.9° it occupied the center of the decayed area, surrounded by *P. digitatum*, which extended up to the margin. The area covered by *O. citri-aurantii* continued to increase at higher temperatures, until at 30.2° and 31.9° it had so enlarged as to occupy the entire decayed surface. The rot was watery and much softer than that produced by *P. digitatum* alone.

The growth of *Oospora citri-aurantii* + *Penicillium italicum* + *P. digitatum* may be compared to that of *P. italicum* + *P. digitatum*. (Table 9 and Table 5.) In lemons *O. citri-aurantii* + *P. italicum* + *P. digitatum* gave decidedly higher rates of growth than did *P. italicum* + *P. digitatum*, the rate being even higher than the sum of *O. citri-aurantii* alone plus the combination *P. italicum* + *P. digitatum*. In oranges the rate of decay was quite similar to that in lemons, except that at the two highest temperatures the growth of *O. citri-aurantii* + *P. italicum* + *P. digitatum* was somewhat less than the sum of the increment of *O. citri-aurantii* alone plus the combination *P. italicum* + *P. digitatum*. The combination of the three organisms produced a reddish discoloration underlying the zone of sporulation of *P. italicum* and other effects similar to those previously described for *P. italicum* + *P. digitatum*, with the following exceptions: *O. citri-aurantii* showed on the surface at the middle of the decayed area at 27.2° C. in both lemons and oranges and was the only fungus that showed at 31.9° C.

The addition of *Oospora citri-aurantii* to *Aspergillus niger* + *Penicillium italicum* + *P. digitatum* appeared to increase the rate of decay on lemons but had no marked effect on oranges. (Table 10.) Figure 10 shows the rates of decay at different temperatures when *Oospora citri-aurantii* and the preceding mixtures were used.

TABLE 10.—Rate of decay at different temperatures of lemons and oranges inoculated with *Oospora citri-aurantii*, *Aspergillus niger*, *Penicillium italicum*, and *P. digitatum* in two combinations

LEMONS								
Inoculum	Days after inoculation	Diameter in millimeters of decayed spots in fruit started at—						
		9.6° C.	14.7° C.	19.3° C.	23° C.	27.3° C.	30.1° C.	32.2° C.
<i>A. niger</i> + <i>P. italicum</i> + <i>P. digitatum</i>	4	16	45	86	97	70	35	45
	6	50	93	* 150	* 150	* 150	54	57
<i>O. citri-aurantii</i> + <i>A. niger</i> + <i>P. italicum</i> + <i>P. digitatum</i>	4	16	49	93	118	110	109	43
	6	77	* 150	* 150	* 150	* 150	* 150	68
ORANGES								
<i>A. niger</i> + <i>P. italicum</i> + <i>P. digitatum</i>	4	13	41	66	89	102	47	50
	6	31	80	* 200	* 200	* 200	62	78
<i>O. citri-aurantii</i> + <i>A. niger</i> + <i>P. italicum</i> + <i>P. digitatum</i>	4	16	38	64	87	101	82	43
	6	46	91	117	* 200	* 200	105	99

* Entire circumference of fruit.

TABLE 11.—Isolation tests from decayed spots in fruits inoculated eight days previously with *Oospora citri-aurantii* + *Penicillium italicum* + *P. digitatum* + *Aspergillus niger*

LEMONS

Temperature (0° C.)	Place in decayed spot from which test was made		
	Advancing margin	Midway from margin to inoculation	Under the inoculation
11.5.....	<i>O. citri-aurantii</i> + <i>P. italicum</i> .	<i>O. citri-aurantii</i> + <i>P. italicum</i>	<i>P. italicum</i> .
15.9.....	<i>O. citri-aurantii</i> + <i>P. digitatum</i> + <i>P. italicum</i> .	do.....	<i>O. citri-aurantii</i> + <i>P. italicum</i> .
19.8.....	<i>O. citri-aurantii</i> + <i>P. digitatum</i> .	do.....	<i>O. citri-aurantii</i> .
23.0.....	<i>P. digitatum</i> .	<i>O. citri-aurantii</i>	Do.
27.2.....	<i>O. citri-aurantii</i>	do.....	Do.
30.2.....	do.....	do.....	<i>O. citri-aurantii</i> + <i>A. niger</i> .
32.2.....	do.....	do.....	<i>O. citri-aurantii</i> .

ORANGES

11.5.....	<i>O. citri-aurantii</i> + <i>P. italicum</i> .	<i>O. citri-aurantii</i> + <i>P. italicum</i>	<i>O. citri-aurantii</i> + <i>P. italicum</i> .
15.9.....	<i>O. citri-aurantii</i> + <i>P. digitatum</i> + <i>P. italicum</i> .	<i>O. citri-aurantii</i> + <i>P. digitatum</i> + <i>P. italicum</i> .	Do.
19.8.....	<i>P. digitatum</i> + <i>O. citri-aurantii</i> .	<i>P. italicum</i> + <i>O. citri-aurantii</i>	<i>P. italicum</i> + <i>O. citri-aurantii</i> .
23.0.....	<i>P. digitatum</i>	do.....	Do.
27.2.....	do.....	do.....	Do.
30.2.....	<i>O. citri-aurantii</i>	<i>O. citri-aurantii</i> + <i>A. niger</i>	<i>A. niger</i> + <i>O. citri-aurantii</i> .
32.2.....	<i>A. niger</i>	<i>A. niger</i>	<i>A. niger</i> .

CULTURAL TESTS FROM TISSUES INSIDE THE FRUIT

At the end of the experiment some fruits from the combination of four organisms just mentioned were used for isolation purposes. The results of these experiments are shown in Table 11. In the lemons at high temperatures *Oospora citri-aurantii* was dominant. This may have been due to the fact that *O. citri-aurantii* attacks lemons more readily than oranges. In oranges *Aspergillus niger* was dominant. In the advancing margin of decay *O. citri-aurantii* was more often present in lemons than in oranges, having been reisolated six times out of seven in lemons and four times out of seven in oranges. Midway from margin to inoculation, *O. citri-aurantii* was found at all temperatures in lemons and at all except the highest in oranges. Under the inoculated wound in lemons *O. citri-aurantii* was found at all except the lowest temperatures and in oranges it was found at all except the highest temperature. *O. citri-aurantii* therefore appeared to dominate in lemons at high temperatures and *A. niger* in oranges at the same temperatures. The green mold, *P. digitatum*, was found only under the advancing margin and only at intermediate temperatures from 15.9° to 27.2° C. The blue mold, *P. italicum*, was found in all isolations in lemons and oranges at 11.5° and 15.9°, but above this was present more often in oranges than in lemons.

COMBINED INOCULATIONS OF *BOTRYTIS CINEREA*, *PENICILLIUM ITALICUM*, AND *P. DIGITATUM* AND OF *B. CINEREA*, *P. ITALICUM*, *P. DIGITATUM*, AND *ASPERGILLUS NIGER*

TEMPERATURE RELATIONS OF *BOTRYTIS CINEREA*

In preliminary tests with *Botrytis cinerea* alone the range of temperature for decay on lemons appeared to be greater than on oranges, probably because the former is a more natural host in the field. In 6 days the range for lemons was from about 2° to 26.8° C. (with an optimum about 22.5°) and for oranges from about 3° to 22.5° (with an optimum about 18.5°) under the same conditions. In both oranges and lemons the spores appeared first at 22.5° in 6 to 8 days after inoculation, at 14.5° in 12 days, and at 9.5° in 14 days. In oranges at 26.5° no spores were present even after 10 days; a small hard brown spot only was produced. No marked difference in color was observed at different temperatures.

TEMPERATURE RELATIONS OF *BOTRYTIS CINEREA* + *PENICILLIUM ITALICUM* + *P. DIGITATUM*, AND OF *B. CINEREA* + *P. ITALICUM* + *P. DIGITATUM* + *ASPERGILLUS NIGER*

Experiments were carried on at each of seven constant temperatures with four groups of lemons and four groups of oranges, with inocula as follows: (1) *Botrytis cinerea*, (2) *B. cinerea* + *Penicillium italicum* + *P. digitatum*, (3) *B. cinerea* + *P. italicum* + *P. digitatum* + *Aspergillus niger*, and (4) check. Each of the first three groups contained 70 lemons and 70 oranges divided into seven lots of 10 lemons and 10 oranges, and a lot was placed at each of the seven temperatures shown in Table 12. The fourth group contained 35 lemons and 35 oranges divided into seven lots of 5 lemons and 5 oranges, distributed as in the other groups.

TABLE 12.—Rate of decay at different temperatures of lemons and oranges inoculated with *Botrytis cinerea* + *Penicillium italicum* + *P. digitatum*, and *B. cinerea* + *P. italicum* + *P. digitatum* + *Aspergillus niger*

LEMONS								
Inoculum	Days after inoculation	Diameter in millimeters of decayed spots on fruit stored at—						
		9.5° C.	14.5° C.	18.5° C.	22.5° C.	26.8° C.	30.1° C.	31.1° C.
<i>B. cinerea</i>	4	0	19	20	22	16	0	0
	6	19	25	37	49	23	0	0
	8	29	46	62	88	33	0	0
	10	48	73	132	149	48	0	0
<i>B. cinerea</i> + <i>P. italicum</i> + <i>P. digitatum</i>	4	10	18	22	67	85	20	0
	6	22	43	64	• 160	• 160	28	0
<i>B. cinerea</i> + <i>P. italicum</i> + <i>P. digitatum</i> + <i>A. niger</i>	4	10	18	62	133	105	39	46
	6	19	53	• 160	• 160	• 160	67	81
ORANGES								
<i>B. cinerea</i>	4	0	0	14	10	0	0	0
	6	9	12	22	13	0	0	0
	8	13	20	37	16	10	0	0
	10	24	31	47	18	10	0	0
<i>B. cinerea</i> + <i>P. italicum</i> + <i>P. digitatum</i>	4	0	10	31	66	89	61	0
	6	11	22	76	125	139	80	10
<i>B. cinerea</i> + <i>P. italicum</i> + <i>P. digitatum</i> + <i>A. niger</i>	4	0	12	32	62	71	34	37
	6	12	28	77	126	124	72	64

* Entire circumference of fruit.

The experiment was performed as previously indicated (p. 164). The growth of *Botrytis cinerea* alone and of the two combinations mentioned above is shown in Table 12. Observations on the decay due to the combination *B. cinerea* + *Penicillium italicum* + *P. digitatum* brought out several interesting points. In oranges at 9.5° C. the color of the decay was typical of *B. cinerea*, but the rot had a soft and watery form rather than the leathery pliability of *Botrytis* rot alone. At 14.5° C., some color due to *Botrytis* appeared after 4 days, followed by the blue spores of *P. italicum* in the center and mycelium of *P. digitatum* in the advancing margin after 8 days. At 18.5° *P. digitatum* was still more prominent, occupying 20 mm. on the advancing margin. At 22.5° *P. italicum* was reduced to a few millimeters around the inoculation wound, followed by *Botrytis* for 20 mm. and then *P. digitatum*. At 26.8° the *Botrytis* color was no longer seen on the outside, but when the fruit was cut through the inoculation wound, some of the brown color was found underneath. At 31.1° no growth of any of the three combined parasites was seen. The surface manifestations on lemons were similar to those on oranges. From about 22° to 30° *B. cinerea* + *P. italicum* + *P. digitatum* caused a higher rate of decay in lemons than *B. cinerea* alone or *P. italicum* + *P. digitatum*. (Compare Table 5 with Table 12.) At other temperatures the presence of *B. cinerea* seemed partially to inhibit the growth of *P. italicum* and *P. digitatum*. In oranges this inhibition extended to all temperatures.

From 9.5° to 26.8° C. no difference was found in the external growth of the combination *Botrytis cinerea* + *Penicillium italicum* + *P. digitatum* + *Aspergillus niger* as compared with that of the other combination shown in Table 12 until *Aspergillus niger* appeared. Four days after the inoculation, black spores of *A. niger* were present at 26.8° on oranges and lemons in the center of the decayed area, followed by *Penicillium italicum*, *B. cinerea*, and finally *P. digitatum* in concentric irregular rings. At 30.1° to 31.1° only *A. niger* was showing on the outside of the fruit.

Comparing the rate of decay of the above combination with that of *Botrytis cinerea* alone or with *Aspergillus niger* + *Penicillium italicum* + *P. digitatum*, a decrease is noted from 9.5° to 18.5° C. and a pronounced increase from 22.5 to 31.1°. In oranges there is the same inhibition at low temperatures; that is from 9.5° to 26.8°, while at higher temperatures a slightly greater rate of decay occurs. The inhibition at lower temperatures seems to have been due to *B. cinerea*. At higher temperatures, favorable to active growth of *A. niger*, the rate of decay was increased by the presence of this fungus. As previously shown, *A. niger* also accelerated the rate of decay when used in combination with *P. italicum* and *P. digitatum*.

COMBINED INOCULATIONS OF ALTERNARIA CITRI, TRICHODERMA LIGNORUM, OOSPORA CITRI-AURANTII, BOTRYTIS CINEREA, PENICILLIUM ITALICUM, P. DIGITATUM AND ASPERGILLUS NIGER

The experiments to be reported here were carried out as previously indicated for each of the seven constant-temperature chambers with five groups of lemons and five groups of oranges, with inocula as follows: (1) *Alternaria citri*, (2) *Trichoderma lignorum*, (3) *Oospora citri-aurantii* + *T. lignorum* + *A. citri* + *Botrytis cinerea*, (4) *O. citri-*

aurantii + *T. lignorum* + *A. citri* + *B. cinerea* + *Penicillium italicum* + *P. digitatum* + *Aspergillus niger*, and (5) check. The first, second, and fifth groups each contained 35 lemons and 35 oranges, divided into seven lots of 5 lemons and 5 oranges, and a lot was placed at each of the seven temperatures shown in Table 13. The third and fourth groups contained 70 lemons and 70 oranges, divided into seven lots of 10 lemons and 10 oranges.

Previous to the use of the combination inoculations, the temperature relations of *Alternaria* and *Trichoderma* used singly were studied.

Alternaria citri will advance only slowly or not at all within perfectly sound immature fruit, but it readily invades mature fruit of low vitality. In lemons this experiment, as indicated in Table 13, showed in six days a minimum temperature for decay of 15.2° C., an optimum near 23.2° and a maximum near 27.6°. A longer period would have extended this range. In oranges no growth was observed during the short period of the experiment. Previous work (1) has shown that a much longer period would have been required.

Trichoderma lignorum produces a very characteristic form of decay, but one difficult to measure at the beginning because it is slow to show any difference in color or in softness until after it has proceeded for some time. The changes in color subsequently develop very rapidly. The fungus probably first invades the albedo and afterwards grows to and affects the surface over considerable areas all at once. In lemons it produced decay in six days only at 23.2°, 27.6°, and 30.1° C. (Table 13.) In oranges no decay took place in the 6 days of the experiment.

TABLE 13.—Rate of decay at different temperatures of citrus fruits inoculated with combinations of fungi, in comparison with that of fruits inoculated with each fungus alone

LEMONS

Inoculum	Days after inoculation	Diameter in millimeters of decayed spots in fruit stored at temperatures indicated						
		11.5° C.	15.9° C.	19.8° C.	23.5° C.	27.2° C.	30.2° C.	31.9° C.
<i>Oospora citri-aurantii</i>	4	9	10	18	26	33	33	32
	6	16	20	43	49	59	57	46
<i>Botrytis cinerea</i>	4							
	6	0	13	20	22	16	0	0
		19	25	37	49	23	0	0
<i>Alternaria citri</i>	4							
	6	0	0	0	0	0	0	0
<i>Trichoderma lignorum</i>	4	0	0	10	12	10	12	0
	6	0	0	0	0	0	0	0
<i>O. citri-aurantii</i> + <i>B. cinerea</i> + <i>A. citri</i> + <i>T. lignorum</i> .	4	0	0	0	47	72	84	0
	6	10	14	18	38	41	39	37
		14	27	35	63	82	88	53
<i>Aspergillus niger</i> + <i>Penicillium italicum</i> + <i>P. digitatum</i> .	4							
	6	0	45	86	97	70	35	35
<i>A. niger</i> + <i>P. italicum</i> + <i>P. digitatum</i> + <i>A. citri</i> + <i>T. lignorum</i> + <i>B. cinerea</i> + <i>O. citri-aurantii</i> .	4	16	93	150	150	150	54	57
	6	15	28	68	88	74	88	38
		26	100	150	150	150	133	61

* Entire circumference of fruit.

TABLE 13.—Rate of decay at different temperatures of citrus fruits inoculated with combinations of fungi, in comparison with that of fruits inoculated with each fungus alone—Continued

ORANGES

Inoculum	Days after inoculation	Diameter in millimeters of decayed spots in fruit stored at temperatures indicated						
		11.5° C.	15.9° C.	19.8° C.	23.5° C.	27.3° C.	30.2° C.	31.9° C.
O. citri-aurantii	4 6	0 0	0 10	11 27	12 29	21 45	29 55	29 52
		9.5° C.	14.5° C.	18.5° C.	22.5° C.	26.8° C.	30.1° C.	31.1° C.
B. cinerea	4 6	0 9	0 12	14 22	14 22	0 13	0 0	0 0
O. citri-aurantii + B. cinerea + T. lignorum + A. citri ^b								
		9.7° C.	14.7° C.	19.3° C.	23° C.	27.3° C.	30.1° C.	32.2° C.
A. niger + P. italicum + P. digitatum	4 6	0 13	41 80	66 200	89 200	102 200	47 62	50 78
A. niger + P. italicum + P. digitatum + T. lignorum + B. cinerea + A. citri + O. citri-aurantii.	4 6	9 21	23 60	43 96	64 118	72 126	27 45	23 42

^a Entire circumference of fruit.^b No decay from T. lignorum, A. citri or this combination in six days.

The combination of the four fungi, *Oospora citri-aurantii* + *Botrytis cinerea* + *Alternaria citri* + *Trichoderma lignorum*, in lemons (Table 13) shows an increase in rate of decay as compared to that of any of these fungi acting alone at a temperature about 23° C. and higher. The rate of growth of the combination of the seven fungi (Table 13) showed a slight increase over that of *Aspergillus niger* + *Penicillium italicum* + *P. digitatum* only at the three lowest and the two highest temperatures.

In oranges the combination of the seven fungi (Table 13) showed a rate of decay less than that of *Aspergillus niger* + *Penicillium italicum* + *P. digitatum* except at 9.7° C. The external manifestations were quite similar to those noted in the experiment with *Botrytis cinerea* + *P. italicum* + *P. digitatum* + *A. niger* already described.

Isolation tests from decayed spots produced by a mixture of the seven fungi are shown in Table 14. Isolations were made from the albedo region under the surface. As may be seen from this table, *Oospora citri-aurantii* was isolated in every case except one in lemons, and it was the only fungus found at 32.2° C., while in oranges *O. citri-aurantii* was not isolated so often and was displaced by *Aspergillus niger* at 32.2°. In this respect this combination of seven fungi gave an isolation response similar to that of the combination *O. citri-aurantii* + *Penicillium italicum* + *P. digitatum* + *A. niger* of Table 11. *Trichoderma lignorum* was also isolated only under the inoculated wound in both oranges and lemons from 19.3° to 30.1°. *Botrytis cinerea* did not appear in any of the isolation tests.

TABLE 14.—Isolation cultures from decayed spots produced by inoculations with a mixture of *Aspergillus niger*, *Penicillium italicum*, *P. digitatum*, *Alternaria citri*, *Trichoderma lignorum*, *Botrytis cinerea*, and *Oospora citri-aurantii*

LEMONS

Temperature (° C.)	Advancing margin of decay	Place in decayed spots from which tests were made	
		Midway from margin rot to wound	Under the inoculated wound
19.3.....	<i>O. citri-aurantii</i> + <i>Penicillium digitatum</i> .	<i>O. citri-aurantii</i> + <i>P. italicum</i>	<i>O. citri-aurantii</i> + <i>T. lignorum</i> .
20.3.....	<i>P. digitatum</i>	<i>O. citri-aurantii</i>	<i>A. citri</i> + <i>O. citri-aurantii</i> .
27.3.....	<i>O. citri-aurantii</i>	do.....	<i>T. lignorum</i> + <i>O. citri-aurantii</i> .
30.1.....	do.....	do.....	<i>T. lignorum</i> + <i>O. citri-aurantii</i> + <i>A. niger</i> .
32.2.....	do.....	do.....	<i>O. citri-aurantii</i> .

ORANGES

19.3.....	<i>P. digitatum</i> + <i>O. citri-aurantii</i> .	<i>P. italicum</i> + <i>O. citri-aurantii</i>	<i>P. italicum</i> + <i>O. citri-aurantii</i> + <i>T. lignorum</i> .
20.3.....	<i>P. digitatum</i>	do.....	<i>T. lignorum</i> + <i>P. italicum</i> + <i>O. citri-aurantii</i> .
27.3.....	do.....	<i>O. citri-aurantii</i>	<i>P. digitatum</i> + <i>O. citri-aurantii</i> + <i>T. lignorum</i> .
30.1.....	<i>O. citri-aurantii</i>	<i>O. citri-aurantii</i> + <i>A. niger</i>	<i>T. lignorum</i> + <i>A. niger</i> + <i>O. citri-aurantii</i> .
32.2.....	<i>Aspergillus niger</i>	<i>A. niger</i>	<i>A. niger</i> .

COMBINATION EXPERIMENTS AT LOW AND MEDIUM TEMPERATURES

Some of the more important organisms known to produce decay at low or medium temperatures were tested by inoculating fruits with them and placing the fruits in seven different temperature chambers ranging from 3° to 18.3° C. This experiment was begun on July 11, 1927.

Seven groups each containing 35 lemons and 35 oranges were used, with inocula as follows: (1) *Penicillium italicum*, (2) *P. digitatum*, (3) *Botrytis cinerea*, (4) *Oospora citri-aurantii*, (5) *Sclerotinia libertiana*, (6) *P. italicum* + *P. digitatum* + *B. cinerea* + *O. citri-aurantii* + *S. libertiana*, and (7) check. Each group was divided into seven lots of 5 lemons and 5 oranges, and a lot was placed at each of the seven temperatures shown in Table 15. Bits of mycelia instead of spores were used for inoculations with *S. libertiana*.

It was not feasible to combine the rates of decay in this experiment (Table 15) with those shown in Tables 5, 9, and 12 since it was necessary to use fruits of a different degree of maturity because of the difference in the season. The previous experiment was made in February and the last one in July. In most cases in oranges the rates of decay were higher in this experiment than in the earlier ones where a few of the same temperatures were again used. (Compare Tables 5, 9, and 12 with Table 15.) However, with lemons at some of the temperatures there was considerable agreement. The actual rate of decay in any one chamber should be considered of less significance than the differences in rates of decay in relation to temperature as a whole. (Table 15.)

TABLE 15.—Rate of decay at low and medium temperatures of lemons and oranges inoculated with various fungi

LEMONS

Inoculum	Days after inoculation	Diameter in millimeters of decayed spots on fruit stored at—						
		3° C.	7.3° C.	10.2° C.	12.9° C.	16° C.	17.4° C.	18.3° C.
<i>Penicillium italicum</i>	4	0	0	11	20	29	37	41
	6	0	13	24	40	51	70	75
	8	0	26	45	79	73	98	103
<i>P. digitatum</i>	4	0	0	13	44	79	110	138
	6	0	20	42	123	151	150	150
	8	0	66	104	148	• 160	• 160	• 160
<i>Botrytis cinerea</i>	4	0	0	0	10	14	18	16
	6	0	0	13	22	38	27	29
	8	0	12	28	46	72	• 160	92
<i>Oospora citri-aurantii</i>	4	0	0	0	0	17	24	25
	6	0	0	10	22	37	44	48
	8	0	0	22	41	55	54	68
<i>Sclerotinia libertiana</i>	4	0	0	17	10	35	50	34
	6	0	0	22	25	81	122	87
	8	0	15	47	57	128	148	150
<i>P. italicum</i> + <i>P. digitatum</i> + <i>O. citri-aurantii</i> + <i>B. cinerea</i> + <i>S. libertiana</i>	4	0	0	10	17	59	94	136
	6	0	9	20	89	155	155	154
	8	0	20	83	150	• 160	• 160	• 160

ORANGES

<i>P. italicum</i>	4	0	0	13	21	32	33	30
	6	0	11	43	83	117	130	135
	8	0	17	69	113	114	158	160
<i>B. cinerea</i>	4	0	0	0	0	13	12	10
	6	0	0	0	10	15	18	14
	8	0	0	8	15	18	22	18
<i>O. citri-aurantii</i>	4	0	0	0	8	10	17	17
	6	0	16	20	14	15	41	30
	8	0	19	63	30	20	58	40
<i>S. libertiana</i>	4	0	0	0	0	15	10	12
	6	0	0	0	0	25	30	28
	8	0	0	0	0	56	77	• 200
<i>P. italicum</i> + <i>B. cinerea</i> + <i>O. citri-aurantii</i> + <i>S. libertiana</i>	4	0	14	25	40	55	85	80
	6	0	16	46	75	99	136	136
	8	0	25	73	107	136	160	160

• Entire circumference of fruit.

The external manifestations of the fungi not in combination showed no marked differences from those already described in previous experiments except that spores of *Penicillium italicum* appeared at a temperature as low as 7.3° C. after six days. In the combination at 7.3° more *P. italicum* showed outside than did *P. digitatum*; at 10.2° and 12.9° *P. italicum* occurred in the center followed by *Botrytis cinerea* and then by *P. digitatum* up to the advancing margin; at temperatures from 16° to 18° *B. cinerea* was displaced by *Oospora citri-aurantii*. *P. digitatum* was the only fungus isolated from the margin of decay in the fruit of four different chambers.

In lemons the combination of fungi seemed to produce a decided decrease in rate of decay as compared with the fungus producing the highest rate of decay when acting alone. In oranges the combination seemed to hasten slightly the beginning of decay at low temperatures (7° to 10°), but from 13° to 18° it produced about the same rate of decay as did *Penicillium italicum* alone.

COMBINATIONS WITH INOCULUM OF MYCELIUM

An experiment was carried out at each of the seven maintained temperatures with six groups of lemons and six groups of oranges, with inocula as follows: (1) *Diplodia natalensis*, (2) *Dothiorella ribis*, (3) *Phomopsis californica*, (4) *Pythiacystis citrophthora*, (5) *Sclerotinia libertiana*, and (6) a mixture of the five fungi just mentioned. Each group contained 35 lemons and 35 oranges divided into seven lots of 5 lemons and 5 oranges, and a lot placed at each of the temperatures shown in Table 16.

This experiment was performed as previously indicated except for the manner of preparing the inoculum. The mycelia of fungi which fruit less rapidly in culture were transferred to tubes of sterile water and crushed out in very small pieces. A tube of melted glucose-potato agar at 41° C. was transferred to the crushed mycelium and shaken constantly while it was solidifying. A fairly uniform distribution of the inoculum resulted. A small amount of sterile water was added until a gelatinous mixture was obtained. The wound in the fruit made as in previous experiments was filled with this mixture of mycelium by means of a small spatula. The results are given in Table 16.

TABLE 16.—Rate of decay at different temperatures of lemons and oranges inoculated with various fungi alone and in combination

		LEMONS						
Inoculum	Days after inoculation	Diameter in millimeters of decayed spots on fruit stored at—						
		6.5° C.	13.1° C.	18.2° C.	22.8° C.	27.6° C.	30.7° C.	32.8° C.
<i>Diplodia natalensis</i>	4	0	0	21	59	80	155	116
	6	0	16	54	59	154	160	159
	8	0	24	54	155	* 160	* 160	* 160
<i>Dothiorella ribis</i>	4	0	0	0	11	13	10	8
	6	0	9	16	15	14	11	8
	8	0	9	32	32	18	13	8
<i>Phomopsis californica</i>	4	0	0	13	14	0	8	0
	6	0	20	35	34	9	8	0
	8	0	24	50	60	10	8	0
<i>Pythiacystis citrophthora</i>	4	10	32	55	69	89	43	0
	6	15	57	88	139	139	44	0
	8	25	80	118	155	* 160	60	0
<i>Sclerotinia libertiana</i>	4	0	0	32	38	15	10	0
	6	19	37	63	80	60	14	0
	8	23	83	105	120	105	18	0
Mixture of above fungi.....	4	0	19	38	78	155	155	155
	6	0	22	97	155	* 160	* 160	* 160
	8	0	35	155	* 160	* 160	* 160	* 160
		ORANGES						
<i>D. natalensis</i>	4	0	0	11	40	74	101	96
	6	0	0	35	99	126	167	152
	8	0	0	60	* 200	* 200	* 200	* 200
<i>D. ribis</i>	4	0	0	0	10	11	10	11
	6	0	0	12	14	16	12	11
	8	0	0	12	23	22	13	13
<i>P. californica</i> *	4	0	0	23	41	51	26	0
<i>P. citrophthora</i>	6	0	25	54	79	76	27	0
	8	0	32	74	101	114	28	0
<i>S. libertiana</i>	4	0	0	15	15	0	0	0
	6	0	0	55	80	0	0	0
	8	0	0	85	130	0	0	0
Mixture of above fungi.....	4	0	0	33	67	74	* 200	117
	6	0	0	39	110	106	* 200	* 200
	8	0	0	62	* 200	* 200	* 200	* 200

* No decay in 8 days.

* Entire circumference of fruit.

Both in lemons and in oranges the combined inoculations resulted in the complete dominance of the external color and odor characteristic of *Diplodia natalensis*. The rapidity of decay of lemons due to the mixture was less than that of the most rapid organism acting alone at 6.5° and 13.1° C., but the rapidity of decay due to the mixture was greater at higher temperatures. In oranges the combination produced an inhibition at 6.5° and 13.1°, an increase at 30.7° and 32.8°, but showed a decay about equal to that of *D. natalensis* at intermediate temperatures. Using the method previously indicated, the writers made 40 isolations from the combined inoculated fruits, taking the tissue from under the advancing margin, midway from margin to center, and under the inoculated wound. In 38 cases the cultural tests showed the presence of *D. natalensis* alone.

TABLE 18.—Average percentage of the circumference of oranges rotted six and eight days after inoculation with various fungi when kept at temperatures indicated *

Inoculum	Days after inoculation	Percentage of circumference rotted when stored at—																		
		3° C.	6° C.	7° C.	10° C.	13° C.	15° C.	16° C.	18° C.	19° C.	20° C.	21° C.	23° C.	25° C.	27° C.	28° C.	30° C.	31° C.	32° C.	33° C.
<i>Aspergillus niger</i>	6				0		0			0			11.6		24.3			37.7		45.2
	8			0	0		0			8.7			20.8		37.7			100.0		100.0
<i>Diplodia natalensis</i>	6								20.3				57.4							
	8							34.8					100.0							
<i>Oospora citri-aurantii</i>	6	0	0	9.2	8.7		7.5			15.6			16.8		26.1					
	8	0	0	11.0	19.2		11.6			25.5			30.1		45.8				30.1	
<i>Trichoderma lignorum</i>	6				0		0			0			0							
	8				0		0			0			23.2					0		
<i>Pythiacystis citrophthora</i>	6	0	0		14.5				31.3				45.8					43.4		
	8	0	0		18.5				42.9				58.5					66.1		
<i>Penicillium digitatum</i>	6			6.3	24.9						100.0				100.0			60.6		
	8			9.8	40.5													66.1		
<i>P. italicum</i>	6	0	0	7.5	14.5		23.0				34.8				38.2			44.6		
	8	0	0	12.7	21.4						50.4				49.8			47.5		
<i>Dothiorella ribis</i>	6	0	0			0			6.9				8.1					57		
	8	0	0			0			6.9				13.3					12.7		
<i>Sclerotinia libertiana</i>	6	0	0			0			35.2				51.2					6.9		
	8	0	0			0			54.4				83.2					6.9		
<i>Botrytis cinerea</i>	6	0	0	5.2									7.5							
	8	0	0	7.5									9.2							

* No growth after 8 days after inoculation with *Phomopsis californica*.

COMPARISON OF RATES OF DECAY FOR ALL THE FUNGI USED

Tables 17 and 18 show the decay in terms of percentage of the circumference of the fruit rotted at the temperatures and times indicated. The fungi are arranged in a manner to show those that produce decay most readily at higher temperatures as compared with those that produce decay most readily at lower temperatures. The fungi producing decay most rapidly at the higher temperatures, 30° to 33° C., on lemons are *Aspergillus niger*, *Diplodia natalensis*, *Oospora citri-aurantii*, and *Trichoderma lignorum*. The fungi producing decay in eight days at the low temperature of 7° or below are *Pythiacystis citrophthora*, *Sclerotinia libertiana*, *Botrytis cinerea*, *Penicillium digitatum*, and *P. italicum*. All the fungi produce decay readily at the intermediate temperatures.

DISCUSSION AND CONCLUSIONS

The results of these experiments as a whole, although somewhat confusing in certain particulars, appear to justify certain rather definite conclusions.

Some of the striking features to be noted are: (1) The selective effect of temperature, in many cases enabling one organism in a mixture to dominate the others in producing decay; (2) the depressing or accelerating effect of a given mixture on rate of decay as compared with that produced by the most rapidly growing organisms of the mixture when used alone; (3) the influence of given mixtures of organisms on the color and consistency of decay; and (4) the differences in temperature range, and in optimum, maximum, and minimum temperatures of the various organisms for decay during a given time.

The selective effect of temperature was strikingly shown in the mixture of *Aspergillus niger*, *Penicillium digitatum*, and *P. italicum* where *Aspergillus* dominated all the others at the higher temperatures and *P. italicum* at the lower, with graduating mixtures at intermediate temperatures. This selective effect in most cases (not all) appeared to be related to the proximity of the given temperature to the range for optimum growth of the dominant fungus when acting alone. *A. niger* is known to grow well at temperatures above which the two species of *Penicillium* make only a slow growth. *P. italicum*, on the other hand, grows better at lower temperatures than *P. digitatum*, and at such temperatures *A. niger* makes a very feeble growth.

The selective effect also appears to be influenced by the resistance or susceptibility of the host as shown in the mixture *Penicillium digitatum* + *P. italicum* + *Oospora citri-aurantii* + *Aspergillus niger*, where *O. citri-aurantii* dominated at the highest temperature in lemons and *A. niger* in oranges. While lemons and oranges show very little difference in susceptibility to *A. niger*, lemons are much more susceptible to *O. citri-aurantii* than are oranges. In the mixture, therefore, *O. citri-aurantii* was able to dominate *A. niger* in lemons, but not in oranges at the same range of temperature.

The increase or decrease in rate of decay produced by mixtures of fungi as compared to the rate of decay caused by the most rapid organisms alone was marked in some cases, but in many others the

rate of decay was not greatly changed. This increase or decrease varied not only with temperature, but with different combinations of organisms. The mechanism of this depression or acceleration of the mixture is probably related to the combinations of enzymes that are present and their action in making food materials for growth available, or in producing inhibiting substances. The specific food requirements of the respective fungi and the competition which these imply are probably of prime importance in attempting an explanation of the behavior of the fungi in mixtures. Further work on this phase of decay is greatly needed. Qualitative and quantitative study of the enzymes of the fungi is suggested. The most striking example of increase in decay due to the combined effect of organisms is the combination *Oospora citri-aurantii* and *Penicillium digitatum*, where the combined inoculation showed a rate of decay as great as or even greater than the sum of the rates of *O. citri-aurantii* and *P. digitatum* when used separately. Similar effects, though less pronounced, due to other combinations were also evident, such as certain mixtures containing *Diplodia natalensis*. Depression in rate of decay due to mixed inoculations was not so striking, but was evident in a number of cases. The rate of decay caused by a mixture of *P. digitatum* and *P. italicum* was less than that of *P. digitatum* alone except at the highest and lowest temperatures. The presence of *Botrytis cinerea* in certain mixtures also had a retarding effect on decay as compared to the rate of the most rapid organism of the mixture. The influence of mixtures of organisms on the type of decay was often very noticeable. Increase in softness or in ease of puncturing or pliability was noted in mixtures as compared to decay caused by any one component of the mixture acting alone. Color changes on the surface or in the interior were also noted, as for example, the pinkish discoloration produced by a mixed infection of *P. italicum* and *P. digitatum* at certain temperatures. The experiments served to confirm previous observations which indicated that this characteristic may be used to determine whether or not a mixture of the two organisms is present in decayed fruits.

The results also serve to point out in a general way which of the various fruit-decay organisms are most important at the different ranges of temperature in storage. A low-temperature group as contrasted with a high-temperature group was evident. At an intermediate range of temperature some organisms from both the low-temperature and high-temperature groups are seen to be important. It should be pointed out that, if given sufficient time, many of these organisms may produce some decay over much wider ranges than those indicated in these short-time experiments.

The results of this investigation have served to suggest a partial explanation for certain decay phenomena in field, packing houses, and during transit, that were not previously well understood. Mixtures of spores of different organisms are more likely to be present under the usual conditions of handling the fruit than spores of a single organism. The results obtained from mixed inocula, therefore, together with the results from single inoculations, give a better basis for the interpretation of what goes on under commercial conditions.

SUMMARY

Some of the conditions which might cause variations in the rate of decay of citrus fruits, aside from mixture of organisms and temperature, were first considered.

It was determined that a variation in the number of *Penicillium italicum* spores between approximately 125,000 and 2,000,000 per cubic centimeter of inocula made little difference in the rate of decay.

At an air humidity of about 43° and an air temperature of 22.2° C. the rate of decay of citrus fruits inoculated with *Penicillium italicum* was the same whether the inoculum was inserted 3, 6, or 9 days after they were picked. When the fruits were inoculated soon after they were picked, the rate of decay was slightly greater.

It was found that seven lots of mature lemons, each held for two weeks at a different temperature, ranging from 9.8° to 31° C., and then inoculated with *Penicillium italicum* showed no difference in rate of decay due to differences in previous exposure.

Lemon fruits held at temperatures alternating from 9.8° to 27.1° C. every 12 hours showed average rates of decay not far from those of fruits held at a constant intermediate temperature.

An increasing rate of decay related to changes in color of lemons from deep green to deep yellow was found. This increasing rate of decay also corresponded to a decreasing resistance of the rind to a pressure necessary to puncture it.

When inocula containing certain mixtures of spores were used the rate of decay greatly increased; when inocula containing certain other mixtures were used the rate was lower than when any one of the components of the mixture was used alone; and when inocula containing still other mixtures were used the rate approximated that of the most rapid component acting alone. With most mixtures temperature had an important influence on decrease or increase in rate.

A mixed inoculum containing spores of *Penicillium italicum* and *P. digitatum* gave increased rates of decay on lemons at the higher and lower ends of the temperature range. The mixture also produced a reddish discoloration of the decayed tissue.

A mixed inoculum containing *Aspergillus niger*, *Penicillium italicum*, and *P. digitatum* produced at the lower temperatures higher rates of decay than the highest rate of any one of the fungi alone.

Oospora citri-aurantii in mixtures with *Penicillium italicum* and *P. digitatum* caused greatly accelerated rates of decay, especially on lemons, where at low and intermediate temperatures the rate was more than the sum of the rates produced by the fungi alone.

Botrytis cinerea in mixtures with *Penicillium italicum* and *P. digitatum* appeared to have a depressing effect on the rate of decay.

In other mixtures *Botrytis cinerea* appeared as a rule to have a depressing effect at low and intermediate temperatures, *Aspergillus niger* to have an accelerating effect at intermediate and high temperatures, and *Oospora citri-aurantii* to have an accelerating effect at nearly all temperatures.

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COTTON ROOT-ROT INVESTIGATIONS IN ARIZONA¹

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INTRODUCTION

Studies have been conducted during recent years on the cotton root-rot disease at different places in the Southwestern States. These have contributed new information on the habits of the fungus,² its manner of spreading through the soil, its recurrence from year to year, its fruiting behavior, factors influencing its severity, and treatments that check its advance or effect its partial control. During the seasons 1925, 1926, and 1927 further evidence was obtained at the United States Field Station at Sacaton, Ariz., that certain chemical disinfectants and organic manures are effective in reducing the injury and that the fungus is more destructive and more persistent in recurrence at the margins of areas where new territory is being invaded. The behavior of the fungus in cultures on different media, its ability to grow on dead roots, and the relationship of dead roots in carrying over the disease in the soil were also studied. The results of these experiments and observations are reported and discussed in this paper.

USE OF CHEMICAL DISINFECTANTS

The possibility of controlling root rot by the use of disinfectants applied to the soil (fig. 1) has occurred to several investigators, but the results of experiments have not indicated that control by such measures can be made practicable.

The efficiency of formaldehyde for eradicating the disease for small, isolated spots has been reported by the senior writer in previous publications (1, 2).³ Recognizing the advantages afforded under irrigation for applying solutions that should be expected to reach the fungus in all parts of the root zone, the senior writer conducted experiments in 1923 and 1924 irrigating badly infected areas with solutions of formaldehyde. The results of these tests (2) were sufficiently promising to justify further study.

When the tests were continued in 1927 it had become known from previous study that the disease is likely to show more injuries in areas that were only recently involved than in areas that have shown the disease in varying degrees for several years. For this reason plots were selected for treatment on the outside margin of a root-rot area where there had been a recent invasion. A circular area of disease had extended into plot C2-15 about 1919 and by 1925 had crossed into C2-14, a distance of 53 feet. The diseased area in these two plots was measured and charted in 1926. (Fig. 2.) In the spring of 1927 this area, together with the marginal area 4 feet beyond

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² *Phymatotrichum omnivorum* (Shear) Duggar.

³ Reference is made by italic numbers in parentheses to "Literature cited," p. 221.

the arc of the active disease, was checked off into basins about 8 by 8 feet. The basined area on plot C2-14, which included 452 square feet, was treated with carbon bisulphide, applied with the irrigation



FIGURE 1.—A, Attempt to exterminate small isolated spots of root-rot disease in an alfalfa field by perforating the soil around the dead plants with holes and repeatedly filling these with formaldehyde. Only two plants shown in the inner circle of plugged holes had died when the treatment was made, and the disease in this case was arrested, but many similar trials were unsuccessful in preventing further spread through the soil. B, Method of applying disinfectants to infected areas before planting cotton in the spring. Formalin (1 per cent) and cresylic acid (2 per cent) were found to be very effective in preventing the recurrence of the disease where it had extended into new areas

water, 1 part to 100 at the rate of 3 acre-inches. On plot C2-15 an area of 1,903 square feet was basined, and 3 acre-inches of a 2 per cent solution of cresylic acid was applied to 1,080 square feet. To

the remaining 823 square feet a solution of formaldehyde (1-100) was applied, likewise at the rate of 3 acre-inches. (Fig. 1, B.)

The disinfectants were applied on April 22, just after a stand of Pima cotton seedlings had broken through the ground. All of the cotton seedlings were killed by the different solutions, and where the cresylic acid was applied Bermuda grass which had been turned under without being killed was apparently exterminated. The cotton was replanted on April 28, and the new seedlings appeared to be little affected by the disinfection of the soil. Germination, however, was a little slower where the cresylic acid had been applied.

It was apparent early in the season that the carbon-bisulphide treatment on plot C2-14 had not reduced the root rot to any great extent. Several centers of infection appeared in July and August,

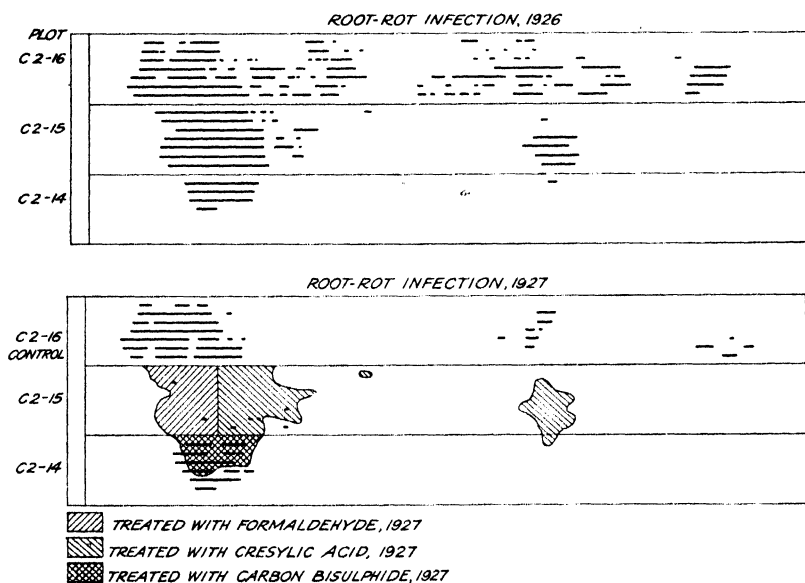


FIGURE 2.—Effects of formaldehyde and cresylic acid on areas recently invaded by the root-rot fungus in plot C2-15. The carbon-bisulphide treatment on plot C2-14 apparently had no effect on the fungus. Infection shown by heavy lines

and by the end of the season the infection was more extensive than in the year previous. On plot C2-15, however, the areas treated with cresylic-acid and formaldehyde solutions showed no infection until late in the season, and only five plants were dead when inspection was made on October 29.

Diagrams showing the extent of the disease in 1926 and 1927 on these two plots and on C2-16, used for control, are shown in Figure 2. Although the extent of infection on control plot C2-16 was less in 1927 than in the year previous, it will be noted that the three principal centers of infection still persisted, whereas in plot C2-15 one of the two principal spots of infection disappeared entirely, and the other showed no evidence of the disease until late in the season, when a few small spots appeared and six or eight plants were killed before the first frost occurred.

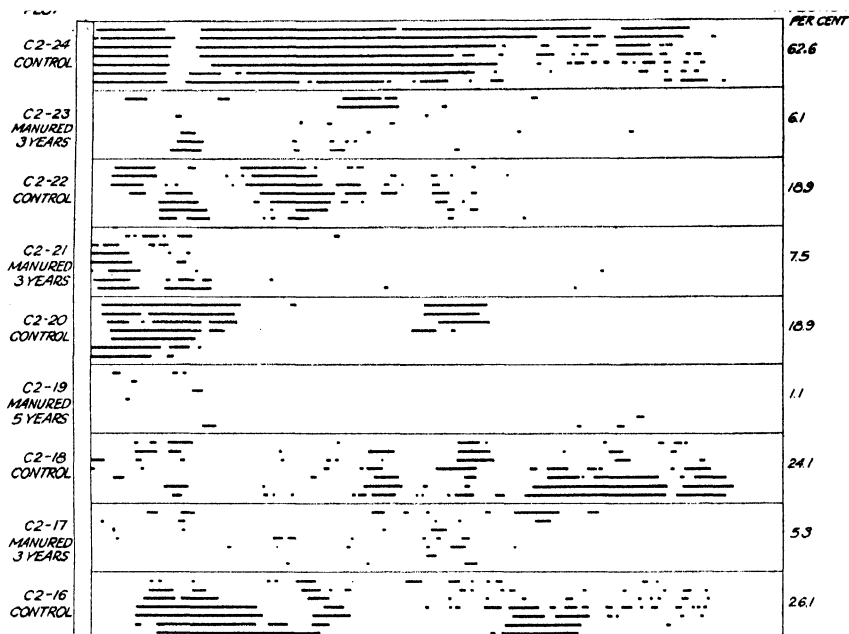


FIGURE 3.—Area infected by root rot (heavy lines) in quarter-acre experimental plots at the end of the season of 1925. Plots C2-17, C2-21, and C2-23 had received applications of organic materials for three years and plot C2-19 for five years

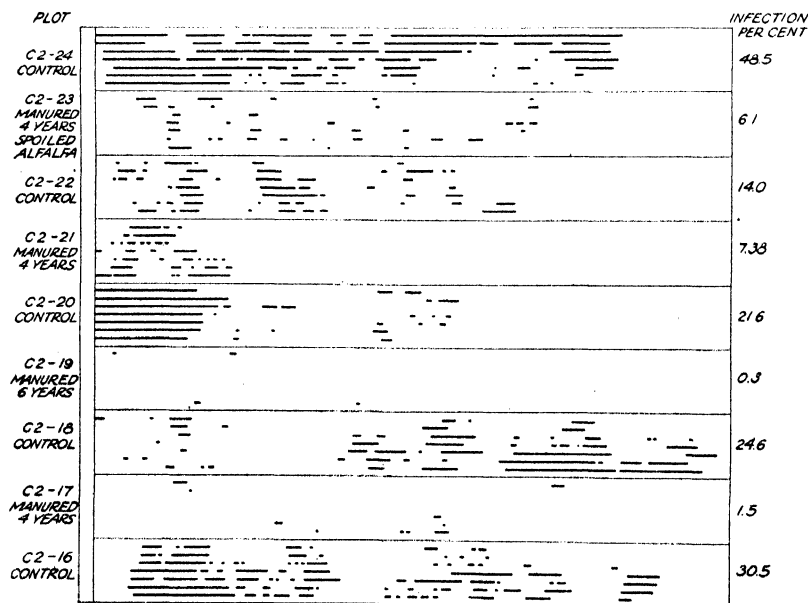


FIGURE 4.—Area infected by root rot (heavy lines) in quarter-acre experimental plots at the end of the season of 1926. Plots C2-17, C2-21, and C2-23 had received applications of organic materials for four years and plot C2-19 for six years

CONTROL EXPERIMENTS WITH ORGANIC MANURES

The effects of farmyard manure and rotted alfalfa on the occurrence of the root-rot disease in certain areas at the United States Field Station at Sacaton, Ariz., have been reported by King and Loomis (4) for the years 1921 to 1924. The experiments were continued during 1925, 1926, and 1927, the farmyard manure being applied in furrows to plots C2-17, C2-19, and C2-21, and rotted alfalfa to plot C2-23, and the alternate plots C2-16, C2-18, C2-20, C2-22, and C2-24 receiving no treatment. Seed of the Pima (Egyptian) cotton was planted in rows directly over the trenches of buried organic matter. At the end of each season, just before the frost period, the locations of all

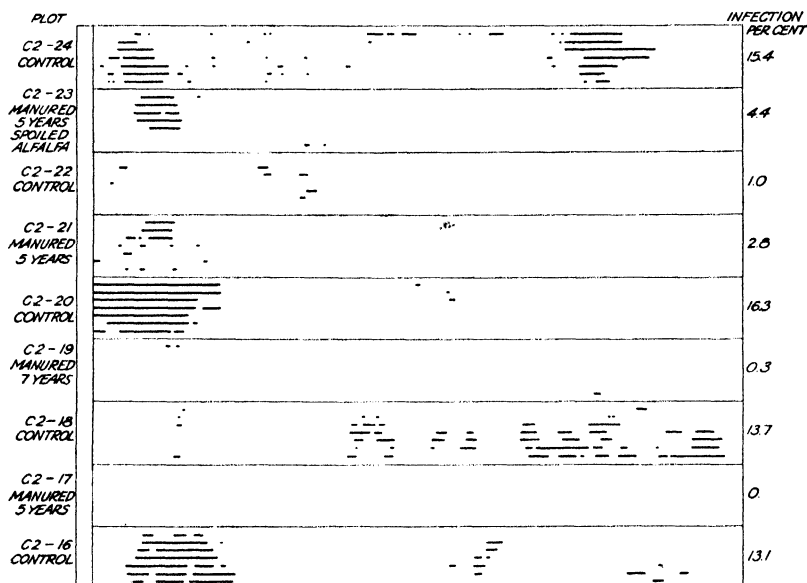


FIGURE 5.—Area infected by root rot (heavy lines) in quarter-acre experimental plots at the end of the season of 1927. Plots C2-17, C2-21, and C2-23 had received applications of organic materials for five years and plot C2-19 for seven years

dead plants were determined by measurements, and maps were prepared showing diseased areas. These maps constitute Figures 3 to 5.

At intervals of two weeks during the period when plants were being killed by the disease the number of dead plants in each plot was recorded. These data are shown in Table 1.

It will be noted from the diagrams that the infection had been reduced generally throughout all of the nine plots, but the reduction was much greater on plots where the organic manures were applied. As previously reported (4), the extent of infection in C2-17 in 1922 included 65.6 per cent of the area, and the infection in C2-19 in 1919 included 71.7 per cent of the area. In 1927 plot C2-17 was entirely free from the disease after five years of the manure treatment, and only 0.03 per cent of the area of plot C2-19 was infected after seven years of treatment.

TABLE 1.—Number of cotton plants killed by the root-rot disease during intervals of two weeks on $\frac{1}{4}$ -acre manured and control plots^a at the United States Field Station, Sacaton, Ariz., in 1925, 1926, and 1927

Year, plot, and treatment	Prior to Aug. 1	Aug. 15	Sept. 1	Sept. 15	Oct. 1	Oct. 16	Oct. 22	Nov. 10	Total dead plants
1925									
C2-24, control.....	258	612	438	354	254	45	-----	-----	1, 961
C2-23 (manured ^b 3 years).....	15	32	24	57	12	11	-----	-----	151
C2-22, control.....	52	131	136	119	76	10	-----	-----	524
C2-21 (manured 3 years).....	3	13	26	83	37	3	-----	-----	165
C2-20, control.....	52	152	147	141	88	13	-----	-----	593
C2-19 (manured 5 years).....	10	3	6	24	7	2	-----	-----	52
C2-18, control.....	76	224	188	166	75	40	-----	-----	769
C2-17 (manured 3 years).....	3	12	15	36	15	7	-----	-----	88
C2-16, control.....	83	209	206	202	112	82	-----	-----	894
Year, plot, and treatment	July 27	Aug. 10	Aug. 24	Sept. 8	Sept. 22	Oct. 7	Oct. 22	Nov. 10	Total dead plants
1926									
C2-24, control.....	17	89	116	171	330	129	28	34	914
C2-23 (manured ^b 4 years).....	1	18	14	9	55	50	5	1	153
C2-22, control.....	12	22	36	50	75	65	11	12	283
C2-21 (manured 4 years).....	5	10	19	8	84	26	11	2	165
C2-20, control.....	15	114	182	111	162	37	7	3	631
C2-19 (manured 6 years).....	0	1	1	2	8	5	0	0	17
C2-18, control.....	42	81	89	82	180	74	23	10	581
C2-17 (manured 4 years).....	3	2	2	1	11	18	2	0	39
C2-16, control.....	17	54	107	104	268	91	46	15	702
Year, plot, and treatment	July 28	Aug. 11	Aug. 25	Sept. 8	Sept. 23	Oct. 6	Oct. 20	Nov. 3	Total dead plants
1927									
C2-24, control.....	13	27	41	84	121	67	60	0	413
C2-23 (manured ^b 5 years).....	12	14	20	39	31	7	4	0	127
C2-22, control.....	2	2	0	5	6	5	25	0	45
C2-21 (manured 5 years).....	1	0	1	9	26	11	14	0	62
C2-20, control.....	4	3	35	164	139	33	11	0	389
C2-19 (manured 7 years).....	0	0	0	0	1	3	3	0	7
C2-18, control.....	46	43	36	92	78	57	49	0	401
C2-17 (manured 5 years).....	0	0	0	0	0	0	0	0	0
C2-16, control.....	28	23	58	82	108	68	32	3	402

^a The number of plants per plot was approximately 2,600.

^b Spoiled alfalfa.

The breaking up of infected areas without the influence of cultural treatment in places where the disease has been more or less continuous for several years is strikingly shown by the behavior on plot C2-24 during the years 1925 to 1927. This large area of almost solid infection began to break up in 1926, and in 1927 the dead plants were widely scattered except near the extreme ends, where solid spots of considerable size remained. The same phenomenon is also shown in plot C2-22, where in 1919 nearly 70 per cent of the total area was diseased (4) and in 1927 only 1 per cent. (Fig. 4.)

There appears to be no reason to doubt that the treatment with organic manures was effective in reducing plant losses. (Fig. 6.) Also, it may be considered that the reduction of infection on the treated plots had some effect in causing the general reduction throughout the entire series. In most cases where observations have been made over a long period of years with no control measures attempted there has been a tendency for the disease to become more extensive

and more damaging in infected areas that are planted successively to cotton. In the nonirrigated cotton areas the proportion of the plants killed each season may be very variable because of the varying summer

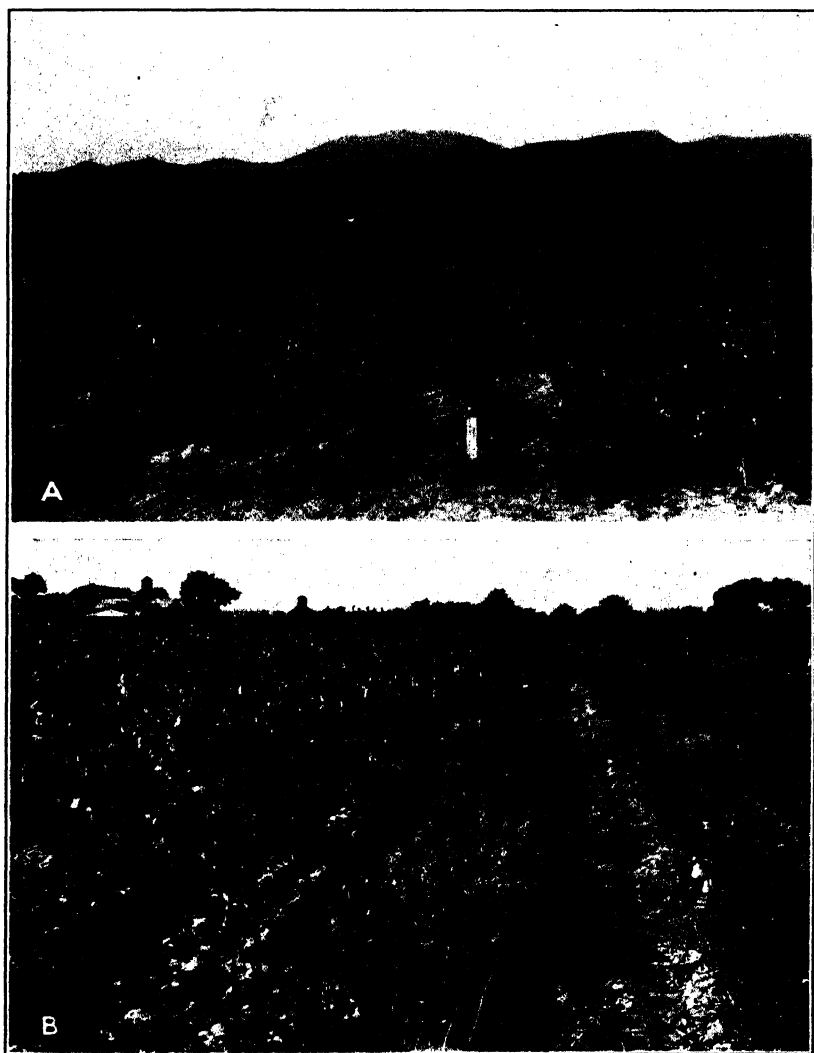


FIGURE 6.—A, Effect of manuring on the occurrence of root-rot disease in cotton. The 7-row plot in the foreground had received no manure, while the plot on the right was manured for four years and the plot on the left for six years. Note the effectiveness of the outside rows of the treated plots as barriers against invasion of the disease from the outside. B, Effectiveness of manure applied in trenches as a barrier against the spread of the root-rot disease. All of the plants in the two rows in the right foreground are dead over a length of 100 feet, while the plants in the adjoining row on the left, where the manure had been applied, remained healthy.

rainfall, but as a rule the disease becomes more destructive the longer it has access to a susceptible crop. The gradual decrease in the extent of disease in the entire area, including treated and control plots, is shown in Table 2.

TABLE 2.—Average percentage of area infected with the root-rot disease on alternate control and manured $\frac{1}{4}$ -acre plots at the United States Field Station, Sacaton, Ariz., 1919–1927

Plots	1919	1920	1921	1922	1923	1924	1925	1926	1927
Control plots: C2-16, C2-18, C2-20, C2-22, and C2-24.....	59.7	57.3	32.2	44.3	23.3	28.8	30.1	27.8	11.9
Manured plots: C2-17, C2-19, C2-21, and C2-23.....			*13.8	*14.1	10.0	8.5	5.0	3.8	1.9

* Only C2-19 treated in 1921 and 1922.

The highest percentage of infection occurred in 1919, when 59.7 per cent of the area was involved. In 1927 only 11.9 per cent of the total area in the five control plots was affected by the disease and only 1.9 per cent of the area in the manured plots. It seems reasonable to suppose that restricting the growth of the fungus in part of the plots, which were only 26.5 feet in width, also resulted in restricting the progress of the mycelium into new ground where it would have been more destructive. Apparently by confining it to rather definite bounds a reduction in losses was obtained.

It is apparent from the data shown in Table 1 that the disease was later in effecting injury to the cotton plants in plots treated with organic manures than where no treatment was given. In 1926 only 9 plants had died on the four manured plots prior to July 27, while 103 plants had died on the five control plots.

Considering our present knowledge of the rate of spread of the disease in cotton fields (3, 5), which in Arizona varies between 12 and 20 feet per season, it is interesting to note the manner in which the advance of the disease was halted when it reached the outside rows of the manured plots. (Fig. 6.) From Figure 5, showing the extent of the infection in 1927, it will be noted that row 7 on plot C2-17 was exposed to nearly 100 feet of infection on the adjacent row of control plot C2-18, less than $4\frac{1}{2}$ feet away, yet no plants on row 7 died. Row 1 on the same plot was exposed to about 20 feet of infection from the adjacent row on control plot C2-16, but there was no evidence of injury to the plants on row 1. On C2-19 the only infection that occurred during the years 1926 and 1927 was located on outside rows 1 and 7 (figs. 4 and 5), which indicates that the mycelium invaded these rows from the control plots but was unable to make rapid progress. A number of excavations were made in plots C2-17 and C2-19 at the end of the season in 1926 and 1927 to determine whether any of the roots of the plants, apparently healthy, had been attacked by the fungus, but all of them were free from root-rot lesions and mycelium. It appears, therefore, that the disease which was once very prevalent in these two plots had been practically eradicated.

TRENCHING EXPERIMENTS

In the effort to determine the method by which the organism moves through the soil in its circular advance from a center of infection, a trench about 20 inches deep was dug about 18 inches in advance of the circumference on one side of a rapidly spreading root-rot spot in an alfalfa field on August 22, 1927. About three weeks after the trench was dug several spore mats appeared on the outside vertical wall near the bottom of the trench. (Fig. 7.) A few days



FIGURE 7.—Conidial mats of *Phymatotrichum omnivorum*, showing as a line of irregular white spots along the bottom of the outside wall of a trench surrounding a root-rot circle in an alfalfa field. This trench, which was dug 20 inches deep and 18 inches in advance of the dying plants, was only partially effective as a barrier, since the mycelium apparently passed under it in two places

later, following a rainy period, the bottom and sides of the trench for several feet became lined with conidial mats. Peltier, King, and Samson (6) showed that the mycelium was seldom found on the roots of healthy alfalfa plants more than 12 inches in advance of the ring of recently wilted alfalfa plants. Since the outside wall of the trench was more than 2 feet beyond the ring of wilted plants, it seems probable that the organism was able to pass under the trench. From near-by excavations it was observed that the mycelium often covered the roots at a depth of 30 inches underground.⁴ Thus it seems probable that some of the mycelium below the 20-inch level may have extended through the soil under the trench and carried the infection to the outside of the barrier. At the end of the season two small spots of infection were found on the outside of the trench, but at the ends of the trench the disease had advanced much farther; hence it was evident that the trench had delayed its progress.

DORMANT CENTERS OF INFECTION

So far there has been no evidence to indicate that root-rot infection could be started in new areas by the dissemination of spores, although spores are produced in great abundance in some places. There has been evidence, however, that infected spots may disappear for a period and then reappear in the same location in later years. This occurrence was illustrated on plots C3-5, C3-6, and C3-7. In 1920 and 1921 the infected areas on these plots which were planted to cotton were charted. (Figs. 8 and 9.) In 1921 several scattered spots on the north end of these plots which were mapped in 1920 did not reappear. During the period 1922 to 1925 the plots were planted to nonsusceptible crops—grain, corn, and sorghums. Two crops were grown each year, which required breaking the land twice a year, in addition to several cultivations that the corn and grain sorghums received. In 1926 the area was again planted to cotton, and the root-rot injuries were charted at the end of the season. (Fig. 10.) Since this infection was not extensive, it was decided to replant to cotton in 1927. The disease was much more destructive in 1927, and in addition to the areas being more extensive than in 1926, small spots appeared where no injuries had appeared in the previous season. (Fig. 11.) This was shown on the north end of plots 5, 6, and 7, where no infection was present in 1921 or 1926, but several spots occurred in 1927. By referring to Figure 8 it will be seen that the area occupied by these spots was infected in 1920. A somewhat similar behavior occurred on plots C3-10 and C3-11, which were also cropped to cereals and sorghums during the years 1922-1925. Several isolated spots appeared at the north end of these plots in 1927 which were not apparent in the cotton planted in 1926, but which were in close proximity to the location of spots that had been mapped in 1921.

Excavations were made in one of these spots in December, 1927, to determine whether any living root fragments remained in the soil from previous crops. At a depth of 18 inches pieces of live roots from the 1926 plants were found, but for the most part the roots of previous crops were badly disintegrated. Some old strands of myce-

⁴ In 1928 rotted roots of pistache and almond trees, covered with root-rot strands, were found in trenches at a depth of 5 feet 8 inches.

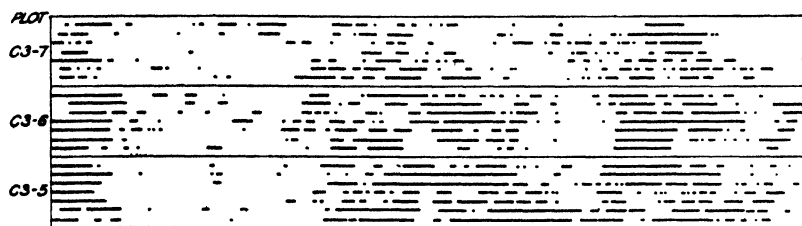


FIGURE 8.—Extent of root-rot infection (heavy lines) in cotton on quarter-acre plots C3-5 to C3-7 in 1920

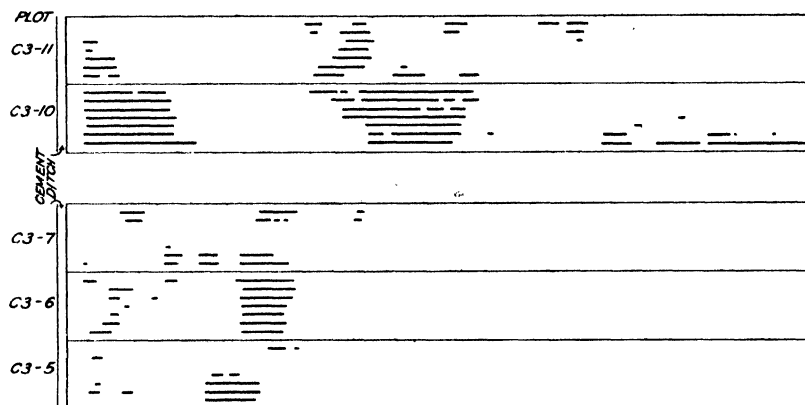


FIGURE 9.—Extent of root-rot infection (heavy lines) in cotton on quarter-acre plots C3-5, C3-6, C3-7, C3-10, and C3-11 in 1921. It will be observed that the infection in plots C3-5, C3-6, and C3-7 was much less than in 1920 as shown by Figure 5

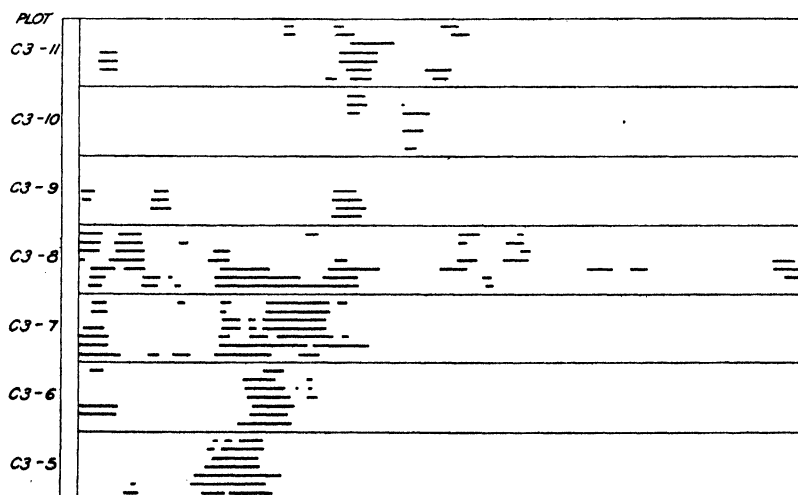


FIGURE 10.—Recurrence of root-rot infection (heavy lines) on quarter-acre plots C3-5 to C3-11 in 1926 after being planted to nonsusceptible crops for four years

lium were found on the old decayed roots and also on the partly decayed roots of the 1927 plants, but they appeared to be dead, and no active mycelium could be found at this time.

INFECTION DEEP-SEATED IN SOME AREAS

The possibility of the fungus being able to exist for long periods at considerable depth was suggested by the reappearance of spots in which the disease had been dormant for some years. That the particular zone of soil in which the disease is most active might vary with the character of the soil and climate was suggested by W. T. Swingle and O. F. Cook. Both predicted that the active zone of the disease recently discovered in a new district in California would likely prove to be deep-seated, on account of the sandy character of the soil and its properties of absorbing heat to considerable depth.

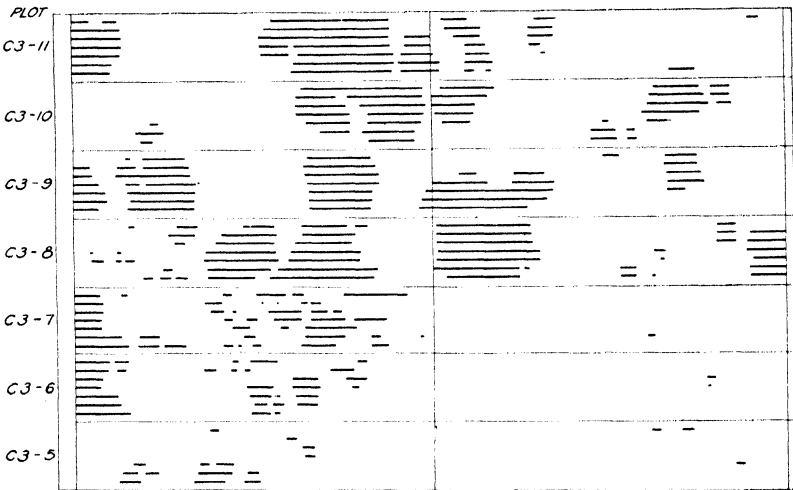


FIGURE 11.—Recurrence of root-rot infection (heavy lines) on quarter-acre plots C3-5 to C3-11 in 1927, the second year in cotton after four years in nonsusceptible crops. Note especially the reappearance of diseased spots in sections of the plots C3-5 to C3-7 where no plants were killed in 1926

Investigations made in December, 1927, in a cotton field in this area where only three cotton plants died during the season proved that this was the case. All of the cotton plants for 10 feet or more surrounding the dead plants were found to be infected, although they showed no evidence of injury aboveground. In almost every case that portion of the taproot extending below the collar for 5 to 10 inches remained uninjured (fig. 12, A), although in some cases mycelium was found on the healthy tissues. Below this the roots showed injuries in varying degrees, but on most plants a section of the taproot 5 to 15 inches in length was completely rotted. Some of these plants with a part of their taproots destroyed had sent out bunches of fibrous roots above the injured area. (Fig. 12, B.)

The activity of the disease at such depths offers a further complication in accurately mapping the extent of the infected areas. It is obvious that under such conditions the extent of the disease is not indicated by the position of the plants that are killed.

CONIDIAL MATS IN ABSENCE OF DEAD PLANTS

Thornber (9), King (3), and others have reported that spore mats are most commonly found in close proximity to plants that have recently died from the root-rot disease. In 1919 one of the writers observed a large spore mat on an irrigation-ditch bank beneath a large cottonwood tree. The tree appeared to be healthy, and the only other vegetation near by was Bermuda grass, which was also healthy in its appearance. The nearest plants that indicated the presence of the root-rot organism were some dead cotton plants about 40 feet away.

In 1927 a field of young alfalfa near Sacaton showed many spots of root-rot infection. The soil was extremely fertile and contained a large quantity of organic matter from decaying roots of native plants and buried cotton stalks of a previous crop. Throughout the summer of 1927 spore mats could be found at almost any time in this field, but they were especially abundant after rainy or humid periods. (Fig. 13.) In a great number of cases large spore mats could be found surrounded by healthy plants and at considerable distance from any dead plants. Since this behavior was unusual, the locations were noted, and later observations showed that the alfalfa plants soon began to die in these areas. Upon digging beneath the spore mats in such locations, large fragments of buried and partly decayed cotton stems or roots were frequently found, and these were invariably densely covered and pervaded with *Phymatotrichum* strands. (Fig. 14, B.)

GROWTH AND DEVELOPMENT OF CONIDIAL MATS

From the behavior of the root-rot fungus when opportunity is afforded for fruiting, it is obvious that the distribution of the mycelium throughout the upper layers of soil is extensive and capable of rapid growth when conditions are favorable. Within a few hours after a summer rain in the vicinity of Sacaton, Ariz., the mycelium in many of the recently infected areas in alfalfa fields rapidly pervades all portions of the soil near the surface and begins to develop an arachnoid structure of whitish mycelium on the surface of the soil about the plants that have recently died. (Fig. 13, A.) This type of mycelium grows rapidly outward from the center where it begins, if conditions remain favorable, and it sometimes covers an area of a square foot or more within 48 hours. The formation of a compact mass of mycelium and conidiophores in a definite palisade structure from 3 to 10 millimeters in thickness rapidly follows the advance of the arachnoid type and develops on top of the thin stromalike structures which form on the surface of the soil. It is not infrequent that several ounces of fungous material is developed on a square foot of soil surface within 8 or 10 hours.

The progressive growth of a small mat is shown in Figure 13. Under some conditions the growth is much more rapid and covers a larger surface than is shown in this instance.

The occurrence of conidial mats in great abundance in areas where a large quantity of organic matter is present in the soil and the rapid rate at which they grow in such places suggest that the organism may derive at least a part of its nourishment from the dead organic material in the soil.

The resistant character of the mycelium which takes part in the development of the spore mats was shown by observations made in the summer of 1927. The bottom and sides of portions of a narrow trench dug for drainage purposes around a large haystack near Sacaton was found to be almost completely lined with large mats on August 16. A period of dry weather followed, and conditions were

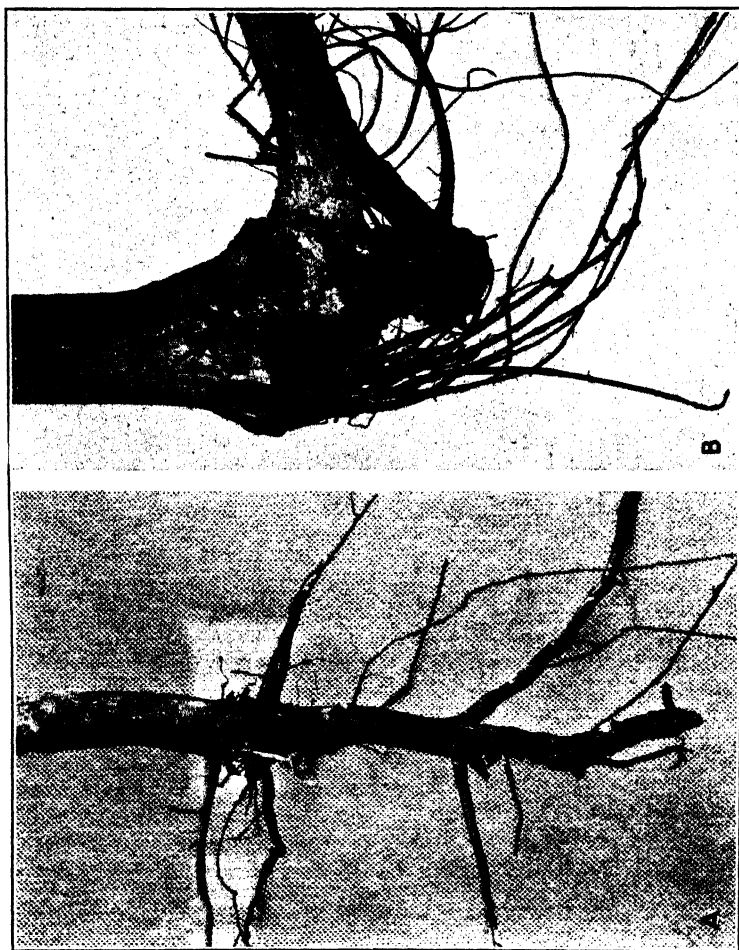


FIGURE 12.—A, Root-rot injury to cotton plant several inches below the surface of the soil. Many large plants in fertile soil are able to survive such injuries for long periods. Under some conditions the mycelium is not destructive to the roots near the surface and large numbers of plants are able to live through the season, although some of the deeper roots are destroyed. B, Fibrous roots produced by cotton plants after the taproot has been destroyed by the root-rot fungus. Note the lateral root at the right which became enlarged because of the injury to the other roots, being responsible for the plant remaining alive until the end of the season.

not again favorable for fruiting until about September 14. At that time the trench was again examined for spore mats, and it was found that many of the old mats, about 29 days old, although badly weathered and disintegrated near the center, had begun new growth on the outer margins, and a whitish elevated band of new material had been formed. A similar occurrence of this revived growth was observed in mats that developed about 18 inches underground in a runway made by rodents. This behavior seems to indicate that in protected locations the fruiting mycelium is capable of existing in a somewhat



FIGURE 13.—Progressive stages in the development of a conidial mat on the periphery of a circle of dying plants in an alfalfa field. A, Photographed at 3 p. m., Sept. 14. Tiny mat 1 day old showing surrounding expanse of filamentous mycelium composed of a large-celled hyphae. B, Photographed at 8 a. m., Sept. 15. Note the growth that occurred during the 17-hour interval. C, Photographed at 8 a. m., Sept. 16. Note how the mat has changed color and become depressed in the center. The growth had practically ceased at this time. (Natural size)

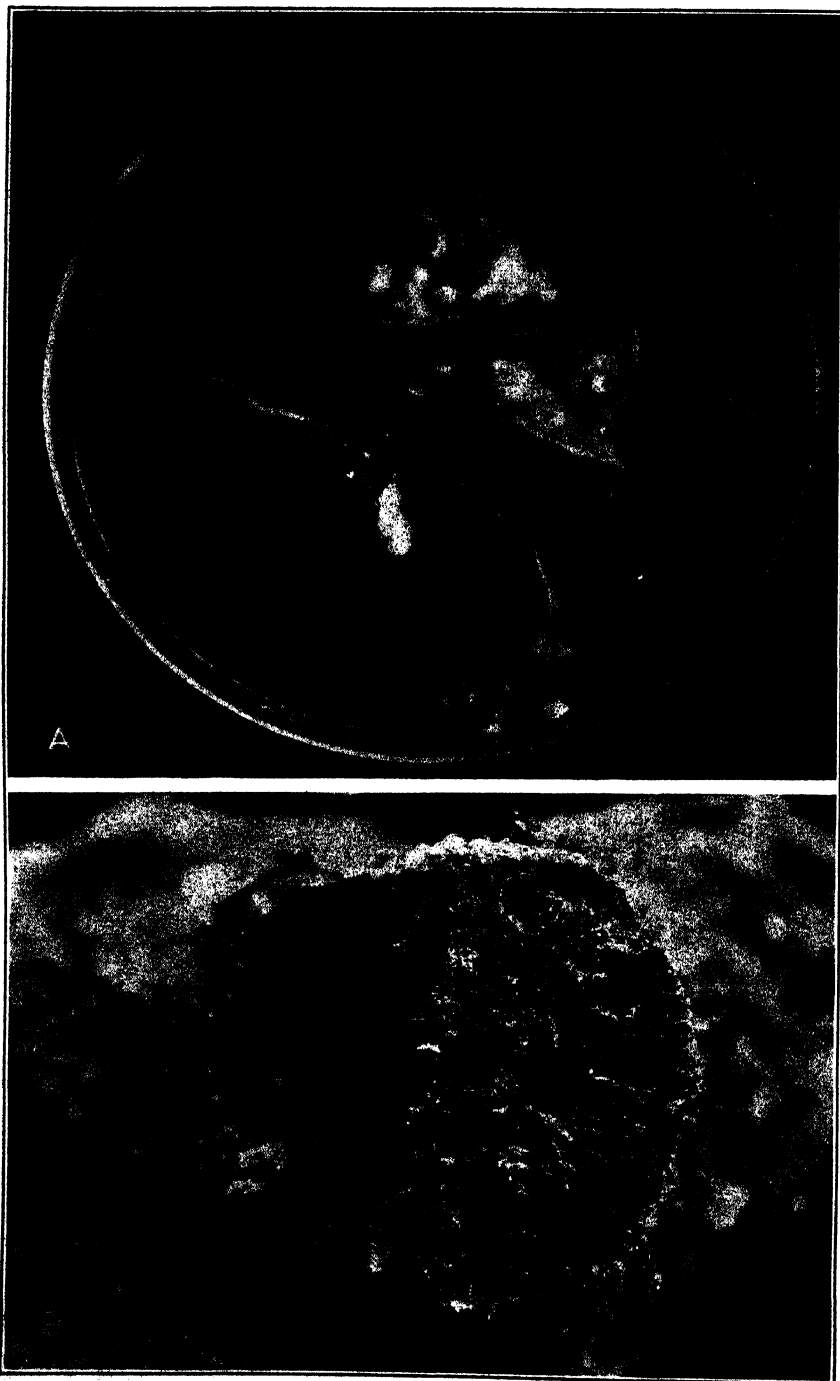


FIGURE 14.—A, Mycelium of *Phymatotrichum omnivorum* growing in culture on old cotton roots that had been buried in the soil for two years after being cut off by the plow. Note the numerous pseudosclerotia that have developed, indicating that the mycelium has been able to draw nutrients from the decayed material. (Natural size.) B, Section of a decayed cotton stalk broken in two to show a large strand of mycelium that had entered through a crack. The stalk had been more than two years below ground.
X 5

dormant condition over long periods and is able to begin new growth when a favorable opportunity is afforded.

The development of spore mats in holes and trenches from 1 to 2 feet below the surface of the soil ⁵ also shows that the distribution of the organism in the soil is extensive and that the fungus is able to take advantage of any favorable situation in which to develop its fruiting bodies.

ARTIFICIAL CULTURES

The root-rot fungus is readily isolated and can be grown on various kinds of culture media. Taubenhaus and Killough (8) and Peltier, King, and Samson (6) have described methods by which isolations can be made. The essential feature of the methods consists in dissecting bits of tissues from just beneath the epidermis on the edge of young lesions on the roots of plants that have been recently attacked. These bits of infected tissue are dipped in a solution of mercuric chloride, 1 to 1,000, for a few seconds, and the excess solution is removed by rinsing them with sterile water or by absorbing the solution between sterile filter papers. The bits of tissue are then dropped on sterile plugs of cotton roots or other nutrient material in test tubes.

In preparing pure cultures for use in inoculation experiments, quart fruit jars were filled with sand or soil and 2-inch sections of cotton roots in alternate layers. After the contents were well moistened with distilled water and the modified caps stoppered with cotton, they were sterilized, and transfers from pure cultures in tubes were added. In a few weeks the contents of many of these jars become permeated with mycelium. In a large proportion of these jars numerous pseudosclerotia were formed, some of which were more than 1 centimeter in diameter. Many small cavities in the sand or soil became completely filled with these pseudosclerotia, whose connection with the large strands was easily determined by examining those on the glass surfaces. In some cases the pseudosclerotia were definitely aerial and were formed on the strands that had grown along the glass above the soil and cotton roots.

It was observed that when the contents of the jars were saturated with moisture no growth of mycelium occurred except on the sections of roots that projected out of the soil. Also when the moisture in the jars became so reduced that no condensation occurred on the glass near the top, it was noted that the growth of the mycelium was restricted. This behavior agrees with field observations in that the moisture content of the soil under natural conditions has an important influence on the growth of the fungus.

When parts of dead cotton roots that had been turned under the soil and had remained buried for two years were dug up, sterilized, and used in the jar cultures, the growth of the mycelium was rapid and profuse, and pseudosclerotia were produced as in the cultures with fresh roots. (Fig. 14, A.) Among the kinds of nutrients on which the fungus was grown successfully were fresh roots of cotton, alfalfa, cowpeas, peach, Lycium, pecan, and dead roots of cotton and alfalfa (fig. 15) 1 and 2 years old. Cultures of the fungus from dead cotton and Lycium roots are shown in Figure 15. Also it was grown suc-

⁵ Spore mats were observed in August, 1928, in trenches at a depth of 5 feet from the surface of the soil.

cessfully on agar media containing decoctions of alfalfa, cotton, beans, prunes, and corn meal, and on cottonseed and wheat kernels. Pseudosclerotia were formed in a majority of the cultures with all of these media. The behavior of the fungus when limited to a nutrient of dead roots indicates that it may be able to exist for long periods under

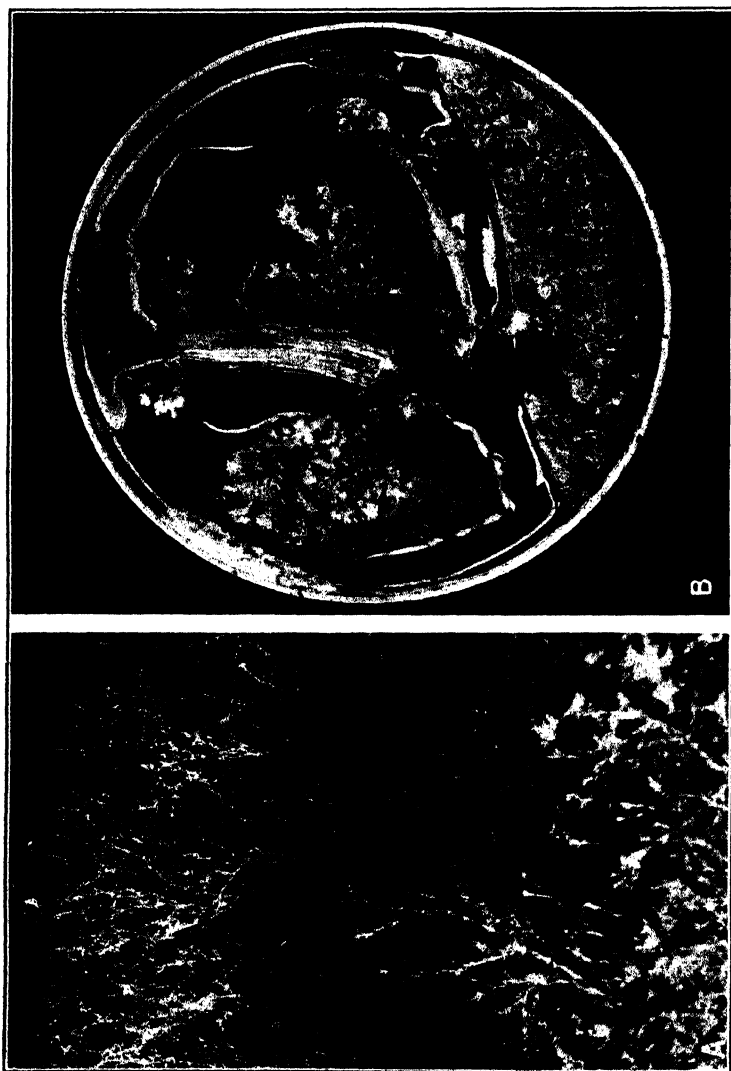


FIGURE 15.—A, Mycelium of *Phymatolichium oenitorum* in pure culture growing on dead cotton roots on a thin layer of prune agar in a Petri dish. Note the thick strands growing from the dead root (out of focus at the bottom of the photograph). The root sections were obtained from several inches beneath the surface of the soil where they had been cut off by the plow and turned under for several months. X nearly 5 diameters. B, Growth of *Phymatolichium oenitorum* on pieces of dead root of *Lycium* sp. on prune agar. The roots became densely covered with the mycelium before it extended to the thin layer of agar. The irregularities in the agar were due to its becoming dried out. A number of small pseudosclerotia are apparent on the mycelium. (Natural size)

natural conditions as a saprophyte. (Fig. 16.) This is in accord with findings of George T. Ratliffe (7) at the San Antonio Field Station in Texas, who found the organism existing on fragments of mesquite roots that had remained under the soil for many years. Not infrequently, alfalfa roots killed by plowing are found long afterward supporting live mycelium. (Fig. 16.)

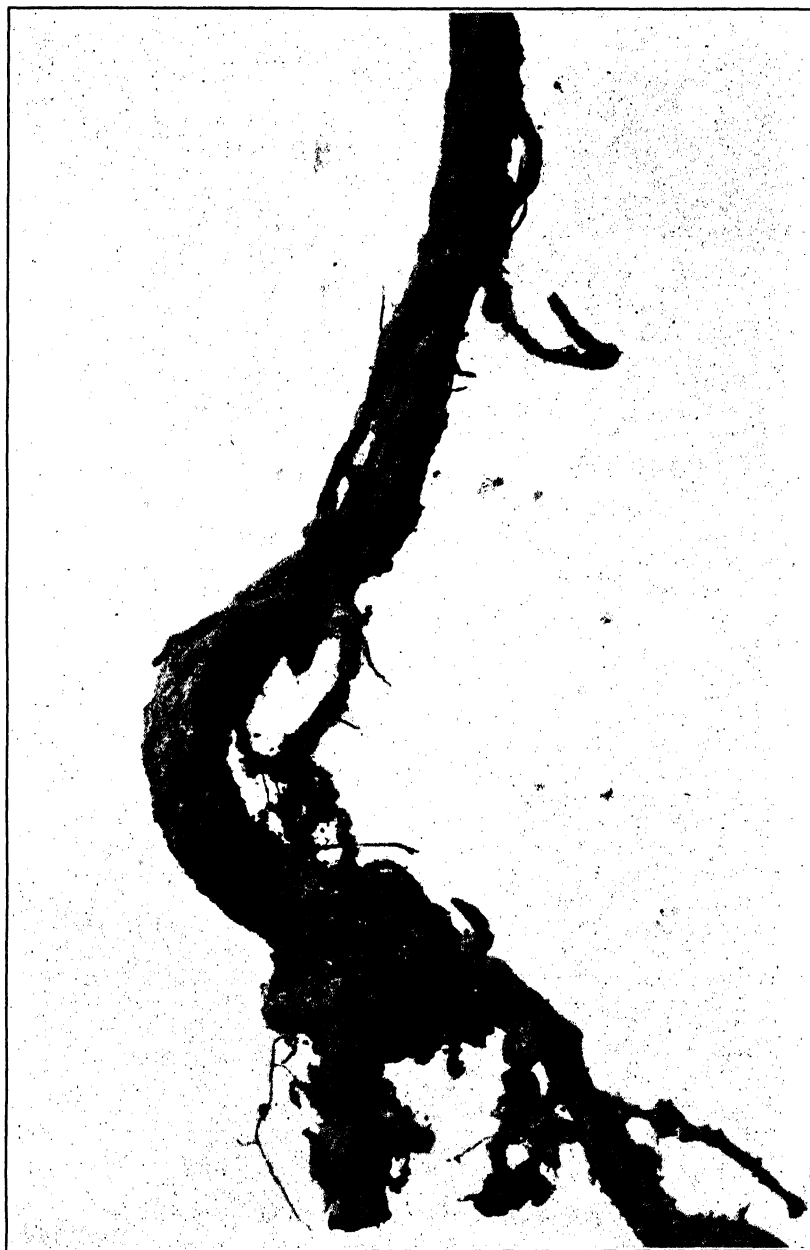


FIGURE 16.—Coarse strands of root-rot mycelium on an old dead alfalfa root. $\times 5$

INOCULATION EXPERIMENTS

The successful inoculation of healthy cotton plants with *Phymatotrichum omnivorum* has been reported by King (1), by Taubenhaus and Killough (8), and by Peltier, King, and Samson (6).

Further experiments in producing infection were conducted during the latter part of the season of 1927 at Sacaton. The methods employed were essentially the same as those described by King (1), in which the taproots of normal plants in uninfected locations were exposed in narrow trenches and the inoculum placed in contact with the healthy roots. The experiments in 1927 demonstrated that inoculations can be made without great difficulty when certain conditions are favorable.

In trench No. 1 the roots of 14 Pima cotton plants of average size were exposed, and sections of roots from recently infected plants were placed in contact with them on September 12. The inoculum was held in place with moist cotton lint, and the trench was refilled with soil. On September 27 one of the inoculated plants wilted suddenly, and two days later two others showed the characteristic symptoms of root-rot attack. (Fig. 17, A.) However, the field was irrigated about this time, and one of the wilted plants recovered and showed no further distress during the season. The second plant that died was dug up, and from it the root-rot fungus was isolated and grown in cultures. None of the other 11 plants exhibited any effects from the treatment, but when they were dug up and the roots examined on December 18 it was found that all of them had been attacked to some extent by the fungus. On several of them a part of the taproot had been completely destroyed, but the plants had been supported by large laterals which were above the infected zone.

In trench No. 2 the roots of 12 very large plants, more than 6 feet in height, were exposed on September 13. Six plants on one side of the trench were inoculated with sections of roots recently attacked by the disease under field conditions, and the six plants on the other side were inoculated with 2-inch sections of cotton roots from pure cultures grown in fruit jars. The sections of infected roots were placed in contact with the taproots of the healthy plants about 4 inches below the surface of the ground. On the twenty-first day after treatment one of the plants inoculated with this pure-culture material wilted slightly and remained in a semiwilted condition for several days. Irrigation water was applied at this time, and the plant recovered and remained healthy until the end of the season. On December 18 the plants were exhumed and their roots examined. It was found that the taproots of all of them were attacked at a distance of 4 or 5 inches below the crown, and in some cases the roots were rotted to a depth of 12 to 14 inches in the soil. The large laterals served to sustain the plants, as only a few of these were injured. When examined in December the healthy surface area of the taproots above and below the rotted area and the surface of many of the laterals were covered by live strands of the root-rot fungus. In some cases the strands had extended 5 or 6 inches beyond the zone of the nearest lesion. This behavior of the mycelium seems to be characteristic during the cool-weather periods in the fall and spring, when the disease is not very active. If these inoculations had been made during the early part of the summer, when the fungus is ordinarily more active, it is believed that a greater

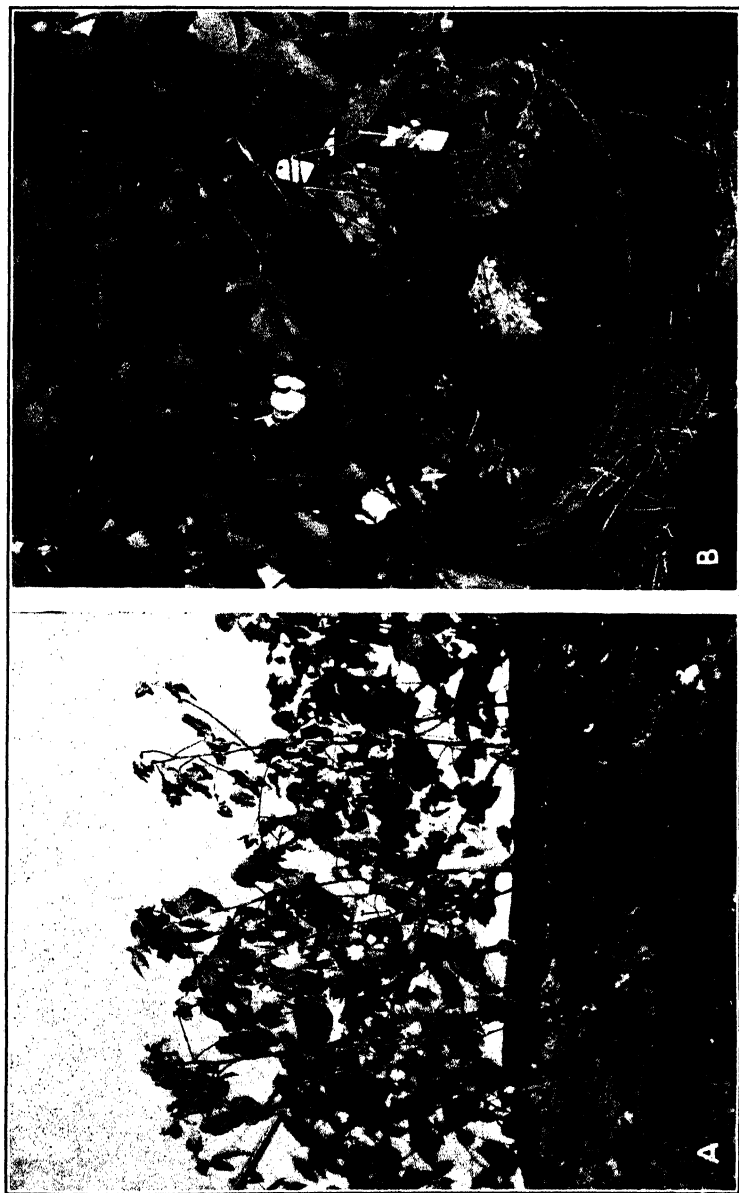


FIGURE 17.—A, Cotton plants dying after being inoculated with the root-rot fungus in an area where the disease had never existed. B, Method of attempts to transmit the root-rot disease to cotton plants grown in drums by use of cylindrical cores of earth from infected spots where the disease was active and fruiting. These efforts in 1927 were unsuccessful.

number of the injured plants would have died.⁶ Even during the summer months, however, roots are sometimes found on which the mycelium is abundant before any lesions or depressions in the root surface appear.

It is apparent from these experiments that infection can be produced in soil areas where the disease has never been in evidence, but the time of the season, the size of the plants and their root systems, and the moisture conditions seem to have an important relation to the progress of the new infection.

Other attempts were made to transmit the disease to healthy cotton plants grown in tanks and drums by inserting cylindrical cores of soil from the active margin of dying spots of alfalfa into the soil adjacent to the plants in the tanks and drums. For the large tanks, cores of soil 12 inches in diameter by 12 inches long with fresh undisturbed spore mats on the upper surface were removed from an infected alfalfa area by the use of date-tree transplanters and inserted in the soil in the tanks between two cotton plants. Smaller cores about 6 inches in diameter by 8 inches long with spore mats on the top surface were used as inoculum for the plants grown in drums. None of the cotton plants died as a result of this treatment, but when the cores of soil were dissected in December live mycelium was still in evidence on partially decayed pieces of alfalfa root. There was no evidence, however, of the mycelium having extended into the soil surrounding the cores, and the greater part of the mycelium was dead and brittle.

SUMMARY

Further studies on the cotton root-rot disease at the United States Field Station, Sacaton, Ariz., have shown that the extension of infection into new areas can be effectively controlled by treating the soil with solutions of formaldehyde and cresylic acid.

Experiments in which organic manures were applied in furrows to alternate $\frac{1}{4}$ -acre plots continuously cropped to cotton for several years show that this treatment is effective in reducing the extent of infection, in delaying the appearance of the disease, and in retarding its injuries to the plants.

The manured plots served as effective barriers in restricting or retarding the advance of the mycelium from adjacent untreated areas.

Plot C2-17, in which 65.6 per cent of the total area was infected in 1922, was entirely free from the disease in 1927 after five years of manuring, and plot C2-19, in which 71.7 per cent of the area was infected in 1920, showed only 0.03 per cent infection in 1927 after seven years of manurial treatment.

The breaking down or dissipation of infected areas where practically all of the cotton plants have died for several years without the influence of any cultural treatment is clearly shown by maps which define the location of the infection over a number of years.

It was indicated that under Arizona conditions the fungus was capable of passing under an open trench 20 inches deep which was dug in advance of a ring of dying alfalfa plants.

⁶ Of 55 cotton plants inoculated July 20, 1928, with one or two 4-inch sections of diseased roots inserted in the soil in contact with the taproot, 15 died within 10 to 25 days and transmitted the disease to adjacent plants. Six plants grown in tanks and drums and inoculated in late July and early August with pure cultures grown in quart fruit jars died within 5 to 19 days, and the disease spread and killed adjacent plants.

The occurrence of conidial mats in areas where no dead plants were evident was verified, and there appeared to be a relationship between these mats and buried fragments of decaying roots beneath them.

A study of maps which define the location of the disease on the same plots over a number of years shows that spots of disease may disappear and recur after a number of years as isolated centers of infection and progress as before.

Further attempts at inoculating healthy cotton plants in fields where no root rot had previously appeared were successful in producing infection on the roots, though only a few of the plants were killed by the disease. This was probably due to the lateness of the season at the time inoculations were made and to the influence of irrigation water on the small mass of mycelium present.

Efforts to transfer the infection to cotton plants growing in large tanks and drums by transferring cylindrical cores of infected soil on which the fungus was fruiting were unsuccessful.

It was found that the root-rot organism is readily isolated and can be cultured successfully on various kinds of roots and other media. Its ability to grow on dead roots indicates that it may exist in nature as a saprophyte and may be able to live in the soil in the absence of live root tissues.

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GRAIN LOSSES IN FEEDING CORN SILAGE TO DAIRY COWS¹

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INTRODUCTION

Dairy cows are able to digest only a portion of the grain in cane and kafir silage and a considerable amount passes into the manure as whole kernels. The practical significance of this loss, which may be attributed to the toughness and small size of the kernels, has been emphasized in a previous publication (2)³. Indications of similar losses were observed with dairy cows that received corn silage. This observation led the authors to investigate the grain losses that actually occur when corn silage is fed to dairy cows.

REVIEW OF LITERATURE

Utilization of whole corn in silage has been investigated briefly by LaMaster and Morrow (10), who made their determinations upon small samples of silage and samples of manure collected from two cows during a 5-day period. The amount of whole corn in the manure, as observed, constituted 1.86 per cent of the grain in the silage. This represents a loss which is relatively small when compared with similar losses (12) of shelled corn which amount to 22.75 per cent with mature dairy cows, 10.77 per cent with yearling heifers, and 6.28 per cent with calves. Wilbur (5, p. 33) found the losses of shelled corn when fed to dairy cows to be between 30 and 35 per cent, while losses of cracked corn were only between 5 and 10 per cent.

Losses which represent 33.91 and 49.46 per cent of the grain in cane and kafir silages, respectively, were observed (2) in the manure of dairy cows. These losses (3) amounted to 9.41 per cent of the calculated total digestible nutrients in kafir silage and 7.13 per cent of those in cane silage. Utilization of the whole grain in kafir silage was from 9 to 13 per cent greater than that of threshed, soaked, and head kafir grain fed to 2-year-old steers (9). LaMaster and Morrow (10) reported 27.55 per cent of the whole grain in cane silage voided in the manure of dairy cows. Cave and Fitch (4) obtained results based on counted kernels in small samples of material, which indicated a loss as great as 90 per cent of the grain in cane silage.

Corn undergoes a loss in the crib as well as in the silo. Corn stored from January 5 to June 1 (147 days) lost 5.6 per cent in weight (6). Gaines (7), from an analysis of grain at the time of harvest, found that corn in the crib lost 2.25 per cent, while kernels in corn silage lost 5.08 per cent of the dry matter. Part of the crude protein

¹ Received for publication Apr. 2, 1929; issued August, 1929.

² Acknowledgement is made to the late Prof. A. C. Baer, and to P. C. McGilliard of the dairy department, who instigated the experimental work comparing corn silage with mangels in dairy rations. Data presented here were derived from cows on this investigation. Kenneth Corbett cared for the cows used in this feeding trial.

³ Reference is made by number (italic) to "Literature cited," p. 227.

and ash of corn kernels (11) was taken up by the plant juices in the silo, thereby increasing the protein content of the leafy portion of the silage. Bailey (1) showed the shrinkage in shelled corn to be due to loss of moisture and carbon dioxide in the respiration of the dormant seed.

There is need of data from more animals over a longer feeding period, which takes separate account of the whole and cut kernels, on which to base a calculation of the losses of corn grain in the silo, and later in the digestive system of the dairy cow.

EXPERIMENTAL METHODS

Four cows on a 20-day feeding trial (10 days preliminary and 10 days experimental) were given a daily ration consisting of 30 pounds of corn silage and 10 pounds of alfalfa hay per 1,000 pounds live weight, and, in addition, mixed grain consisting of wheat bran, ground oats, corn meal, and choice cottonseed meal to meet the Morrison (8, p. 746) feeding standard. Reid Yellow Dent corn cut in the glazed stage of maturity and ensiled in a hollow-tile silo furnished the only source of whole-kernel corn. Samples of the ear corn procured at time of filling the silo were cured on the cob, shelled, and analyzed with the samples mentioned below.

The usual precautions (2) were observed in caring for the animals and recording the amounts of feed eaten and refused. A 10-pound sample of silage was taken from the silo each day during the experimental period. Corn kernels were separated from the silage by hand, weighed, and sampled. The entire amount of corn in any refused feed and in the manure of each cow was separated out daily by use of water and screens. This grain was air dried, weighed, and sampled.

Chemical analyses were made of the shelled corn, corn silage, and grain from the silage and from the manure. The proportion of whole and cut kernels was determined by separating and weighing these fractions from large composite samples of the grain obtained from the silage and from the manure.

PRESENTATION OF DATA

During the 10-day experimental period the four cows consumed 1,335 pounds of corn silage and refused 25 pounds. The ten 10-pound samples of fresh silage contained 24.95 pounds of corn grain. Allowing for 0.78 pound of air-dry grain in the refused silage, the four cows consumed 170.77 pounds of dry matter as grain in the corn silage. By the method described 15.89 pounds of air-dry corn grain (14.48 pounds of dry matter) were recovered from the manure. This loss constituted 8.47 per cent of the grain contained in the silage.

Whole kernels made up 67.87 per cent by weight of the corn grain in the silage. Likewise, 40 per cent of the grain voided in the manure was whole kernels. By applying these percentages, it was calculated that 4.36 per cent by weight of the whole kernels in silage was voided as whole kernels in the manure. It was noticed repeatedly that the starchy portion of numerous cut and broken kernels had been digested, leaving large fragments of corn bran in the manure.

Records of silage consumed and of grain recovered from the manure of individual cows are presented in Table 1.

TABLE 1.—Amount of grain in corn silage fed to dairy cows during 10 days and of air-dry corn recovered from the manure

Cow No.	Silage offered	Silage refused	Moist corn in silage	Air-dry corn in refused feed ¹	Air-dry corn recovered from manure ¹
	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
1.....	300	21	74.85	0.28	2.42
2.....	360	0	89.82	0	6.42
3.....	360	0	89.82	0	4.04
4.....	340	4	84.83	.50	3.01

¹ Since water was used in separating grain from manure and refused feed, it became necessary to report these data on the air-dry basis.

Chemical analyses of the original shelled corn, fresh silage, grain from the silage, and air-dry grain recovered from the manure are presented in Table 2.

TABLE 2.—Composition of original corn kernels, corn silage, and grain from silage fed and of grain recovered from the manure of dairy cows during a 10-day period

Description of sample	Moisture in original material	Composition of dry matter				
		Crude protein	Ether extract	Nitrogen-free extract	Crude fiber	Ash
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Shelled corn cured on the cob.....	9.10	9.62	4.27	82.30	2.53	1.28
Whole kernels from silage.....	49.46	7.14	4.93	84.65	2.38	.90
Whole kernels from manure, air-dry.....	8.74	6.62	4.36	85.59	2.63	.80
Total corn from silage.....	49.46	6.95	4.67	84.72	2.53	1.13
Total corn from manure, air-dry.....	8.90	6.26	2.87	87.30	3.13	.44
Corn silage.....	69.26	8.31	3.45	66.51	17.47	4.26

The changes in composition of the corn grain while in the silo and in contact with the digestive juices of the cow may be obtained from these analyses.

DISCUSSION OF RESULTS

The data presented show that 8.47 per cent by weight of the corn grain fed in silage to dairy cows was voided with the manure. Only 4.36 per cent of the whole kernels in the silage was recovered as whole kernels from the manure. The loss of corn grain in silage was smaller than that with shelled corn (5, 12). These losses were appreciably less than those observed (2) with the small, hard grains in Early Sunrise kafir and Kansas Orange cane silages. The losses of whole corn, as found by this method, are higher than those observed by LaMaster and Morrow (10). There is evidence to show that the corn grain recovered from the manure of these cows is of sufficient value to be salvaged profitably by swine or poultry.

The changes which occurred in the corn grain in the silo and in the cow's digestive system are evident from the analyses presented in Table 2. The apparent increase in the ether-extract content of ensiled kernels is due to the formation of ether-soluble compounds during the fermentation process in the silo. The apparent losses,

as seen in Table 3, were 25.78 per cent of the crude protein, 29.69 per cent of the ash, and 5.94 per cent of the crude fiber in the original whole kernels. Apparent gains were found of 2.85 per cent and 15.45 per cent of the nitrogen-free extract and ether extract, respectively, in the whole kernels separated from the silage. These changes were produced by bacterial and enzymatic action on constituents of the corn kernels, changing them into carbon dioxide and other by-products, some of which were ether-soluble while others appeared as nitrogen-free extract. The loss in ash may be attributed to its solubility in the plant juices, as observed by Perkins (11).

TABLE 3.—Composition of corn grain from silage and from manure compared with that of shelled corn cured on the cob

Description of sample	Crude protein	Ether extract	Nitrogen-free extract	Crude fiber	Ash
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Shelled corn cured on the cob.....	100.00	100.00	100.00	100.00	100.00
Whole kernels from silage.....	74.22	115.45	102.85	94.06	70.31
Whole kernels from manure.....	68.81	102.11	104.00	103.95	62.50
Total corn grain from silage.....	72.35	109.37	102.94	100.00	88.28
Total corn grain from manure.....	65.07	67.21	104.86	123.71	34.38

The corn kernels recovered from the manure had given up a small proportion of their crude protein, ether extract, and ash, and consequently showed a slight apparent gain in content of crude fiber and nitrogen-free extract after coming in contact with the cow's digestive juices. The percentage changes of these constituents in the corn grain are presented in Table 3.

By applying coefficients of digestibility (8, *p.* 722-726) to the nutrients in corn silage and in the grain voided with the manure it was calculated that 5.22 per cent of the digestible crude protein and 5.26 per cent of the total digestible nutrients in the silage were contained in the grain recovered from the manure.

SUMMARY AND CONCLUSIONS

Corn kernels lost protein, crude fiber, and ash in the silo, but gained in percentages of nitrogen-free extract and ether extract.

When dairy cows were fed silage made from dent corn in the glazed stage of maturity 8.47 per cent by weight of the grain in the silage was voided in the manure. Only 4.36 per cent by weight of the whole kernels in the silage was recovered as whole kernels from the manure. Analyses showed slight losses of protein, ether extract, and ash from the corn grain in silage which passed through the cow's digestive tract. Dairy cows utilize the whole and cut kernels in silage more efficiently than they utilize shelled corn.

The corn grain voided in the manure was calculated to contain 5.22 per cent of the digestible crude protein and 5.26 per cent of the total digestible nutrients in the corn silage.

The loss of whole corn in silage fed to dairy cows is decidedly less than that which occurs when cattle consume shelled corn. Even this small loss may be salvaged by allowing swine or poultry access to the manure.

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INFLUENCE OF ATMOSPHERIC AND SOIL MOISTURE UPON SEED SETTING IN RED CLOVER¹

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INTRODUCTION

Red clover (*Trifolium pratense*) is mainly self-sterile, and cross-pollination and fertilization are dependent upon insect visits. The present study was made to determine whether high atmospheric humidity prevents fertilization and whether a deficiency or excess of soil moisture adversely affects the setting of seed. The difficulty of controlling soil moisture, the self-sterility of red clover, the heterogeneity of its genetic constitution, the method of flowering, and the arrangement and number of florets on a head all added to the complexities of the problem involved in this study.

REVIEW OF LITERATURE

The fertilization of red-clover florets is dependent, among other things, on the healthy germination of the pollen, and it has been thought that the stigmatic surface supplies the moisture necessary for this process. A consideration of the germination of pollen under different conditions is of primary importance in this study. Lidforss (3, 4)² has contributed to the knowledge of the germination of pollen of various species.

Hansgird (1), Martin (5), and Westgate and Coe (9) concluded from their experiments that red-clover pollen bursts immediately when immersed in water. The results shown by Williams (10) differ from those of the above-named investigators in that the bursting was not immediate, some grains resisting the action of the water for more than an hour.

Westgate and Coe (9) concluded that pollination did not take place when the flowers were wet. Williams (10) secured fertilization, however, after immersing the pollen grains in water for five minutes and then applying them to the stigmatic surfaces before they had dried, but when they were allowed to dry for an hour no fertilization occurred.

Martin (5) showed that artificial pollen germination is delicately adjusted to the absorption of water. He found that high atmospheric humidity and a germinating medium containing an optimum degree of moisture were essential for germination and concluded that the function of the stigma is to supply water. Martin further suggested that the quantity of water available to the plant may

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² Reference is made by number (italic) to "Literature cited," p. 247.

increase the moisture content of the stigmatic surface, thereby preventing the pollen from functioning properly. The supply of atmospheric moisture would be important if a balance between the water absorbed and that lost by transpiration were necessary for germination.

Westgate and Coe (9) stated that infertile ovules occurred in red clover and that moist soil and atmosphere appeared to be directly correlated with the degree of infertility. These investigators also stated that in first growth many red-clover plants produced 100 per cent infertile ovules. Williams (10) concluded that rainfall and temperature were responsible for a decrease in seed setting in some of his self-pollination studies, but he did not state whether the effect was direct or indirect. Martin (5) also pointed out that the poor seed crop sometimes harvested from the first growth may be related to the abundance of moisture usually present when the first crop is in bloom. While Schlecht (6) failed to obtain a large number of seeds, he concluded that high atmospheric humidity did not prevent the functioning of the pollen.

Welton and Morris (8) studied the effect of rainfall on the vegetative growth of red clover and reported that a positive correlation exists between rainfall and vegetative growth.

GREENHOUSE AND LABORATORY STUDIES

POLLEN GERMINATION

Martin (5) succeeded in germinating red-clover pollen and found that the necessary requirements were a nearly saturated atmosphere and a medium or base containing a specific quantity of water. Various solutions proved to be unsuited for such experiments. His results lack uniformity, which he attributed to the variable physical properties of the base.

An attempt was made by the writer to perfect a technique for testing the germination of red-clover pollen. Pieces of sheet gelatine were soaked in various concentrations of sucrose solution and pollen was placed on them. The results, however, were not consistent. In some cases satisfactory germination was recorded; in others negative results were obtained.

EFFECT OF WATER AND SUCROSE SOLUTIONS ON POLLEN

The results obtained by immersing pollen grains in water and sucrose solutions agree with those of Williams (10) that many grains burst immediately after immersion. Bursting occurred in two ways, by a rapid emergence of the protoplasmic contents through small openings in the cell walls and by a uniform collapsing of the cell. Some of the pollen grains collapsed after they had been in the liquid for a few minutes while others were not affected at the end of 10 minutes. Bursting was retarded to a greater extent on gelatine which had been soaked in a 30 per cent solution of sucrose than in a solution of lower concentration.

EFFECT OF CONTROLLED ATMOSPHERIC AND SOIL MOISTURE ON THE SETTING OF RED-CLOVER SEED

In the greenhouse, flowering red-clover plants afford an opportunity to investigate the viability of the pollen and the receptivity of the stigmatic surface by cross-pollinations. Such a study was carried on under conditions where the atmospheric humidity was controlled and its effect on seed production observed.

In this work, red-clover plants from spring and fall seedings were dug, potted in the field, and transferred to the greenhouse in October, 1926. By the use of artificial illumination, many plants were brought into bloom by the middle of December. Williams (10) has shown that the optimum time for pollination in the field is when the florets are first opened, but under the conditions prevailing in the green-

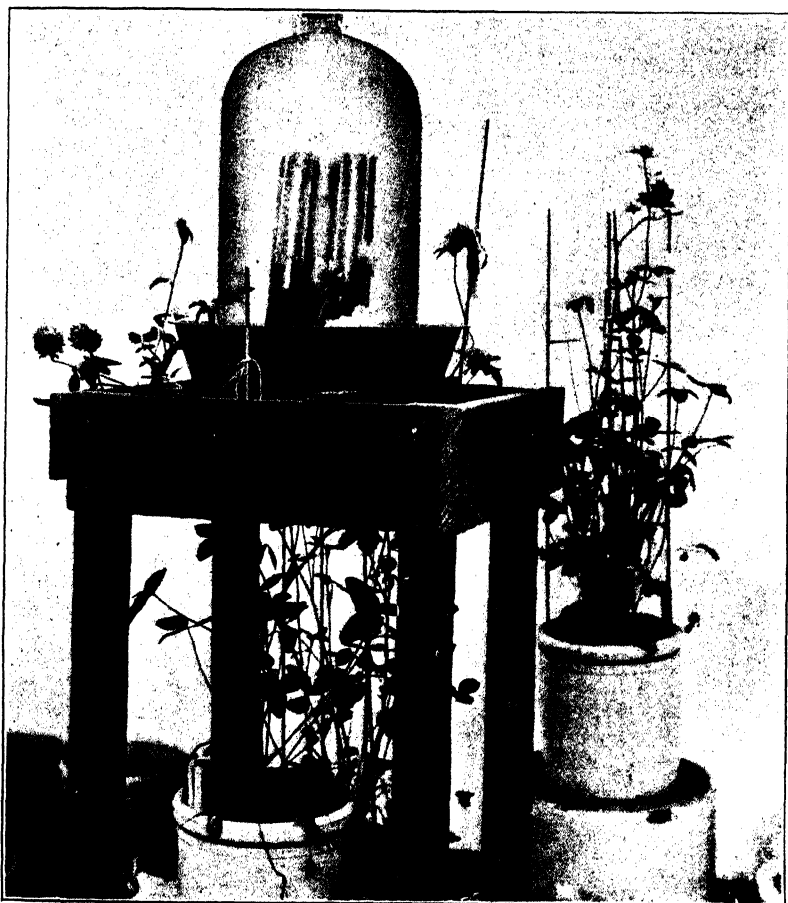


FIGURE 1.—Apparatus used in maintaining high atmospheric humidity for the determination of its effect on seed setting of red-clover plants. Urbana, Ill., 1926

house, the writer found it necessary to wait until the heads were more than one-third in bloom before the flowers were manipulated, as the anthers did not freely dehisce in the early blooming stage.

In order to study the effect of high atmospheric humidity, an apparatus was constructed, consisting of a wooden stand, a wet and dry bulb thermometer, a bell jar, and a tin pan having a depth of 3 and a diameter of 13 inches. (Fig. 1.) Four holes, each $1\frac{1}{2}$ inches in diameter, were cut in the bottom of the pan, and over each hole was soldered a pipe 2 inches long which extended from the bottom of the pan upward. Water placed in this pan for humidification of the

atmosphere did not come in contact with any part of the plant. Flowering stems from different plants were carefully pushed through the pipes, cotton packed around them, and the whole covered with a bell jar. A saturated atmosphere was secured in the bell jar at the end of 10 minutes. The heads of the plants were usually projected into the bell jar 24 hours before pollination. When the effect of soil moisture was to be investigated, the quantity of water supplied to the plants was altered several days before pollination in order that a uniform condition might be established.

Experiments were also conducted under greenhouse conditions where the air was not saturated, but where the relative humidity usually did not vary more than 10 per cent. In cross-pollinating the florets, a small crooked needle roughened with a file proved to be more convenient and effective than camel's-hair brushes, small sticks, needles of the usual types, or the card method used by Williams (10). In tripping the flowers, the needle was inserted between the vexillum and carina. The lower part of the carina was gently pressed with the needle which was pulled upward at the same time. This action tripped the flower, and the pollen was thrown out against the needle which brushed the pistil, thus both gathering and depositing pollen in one stroke. To prevent as far as possible the deposition of pollen from the anthers to the stigma of the same floret, the pollen from the florets of a given plant was permitted to fall on one side of the needle, and the pollen from the other plant similarly on the opposite side. An effort was made to brush the stigmas of the florets in each case with foreign pollen. The time required for the manipulation of the florets of two heads varied from 10 minutes to one hour, depending upon the ease with which the work was done and the number of florets pollinated. The florets were usually manipulated three times at each pollination. After the last pollination, the manipulated heads were ordinarily left in the bell jar at least four days, or until all the corolla tubes had withered.

In these investigations the soil moisture was maintained about as follows: High moisture, where the soil was continually saturated; medium moisture, where a quantity of water was applied which seemed optimum for vigorous growth and the maintenance of a good physical condition of the soil; and low moisture, where the quantity of water available to the plant was barely sufficient to keep it from wilting.

The close proximity of the florets and the fact that it was necessary to carry out the pollinations in as short a time as possible made it difficult to determine accurately the number of florets manipulated on each head. In all, 18 heads were cross-pollinated, and as shown in Table 1, the reciprocal crosses yielded roughly the same number of seeds. When the heads were ripe, the seeds were removed and counted.

After the seed was harvested and the old growth removed, new growth rapidly developed. The plants began to produce flower heads by the latter part of March, 1927. Additional tests similar to those already described were conducted after a sufficient number of flower heads had developed. (Table 2.) In the second tests, the plants were not illuminated at night. To prevent the occurrence of high temperatures within the bell jar, it was necessary to place a shade over the apparatus when the sun was shining.

TABLE 1.—*Effect of atmospheric and soil moisture on the seed setting of red clover, artificially cross-pollinated under controlled greenhouse conditions at Urbana, Ill., December and January, 1926-27*

Plant letters and head numbers		Moisture		Average temperature	Times pollinated	Seeds set
Female parent	Male parent	Soil	Atmospheric, average relative humidity			
			Per cent	° F.	Number	Number
L-1	G-2	High	100	70	1	
G-2	L-1	do	100	70	1	
C-4	Selfed	do	100	70	2	
C-5	C-6	do	100	70	1	
C-6	C-5	do	100	70	1	
G-1	H-1	do	55	65	1	
H-1	G-1	do	55	65	1	
A-1	B-1	Medium	100	70	1	
B-1	A-1	do	100	70	1	
C-1	D-1	do	53	65	1	
D-1	C-1	do	53	65	1	
E-1	C-2	do	53	65	1	
C-2	E-1	do	53	65	1	
F-1	E-2	do	53	65	1	
E-2	F-1	do	53	65	1	
F-2	I-1	do	53	65	1	
I-1	F-2	do	53	65	1	
E-3	I-2	do	53	65	1	
I-2	E-3	do	53	65	1	
B-2	Selfed	do	53	65	2	

^a Head became wilted and browned immediately after pollination, probably the result of rough manipulation.

^b Plants accidentally frosted before seed was formed.

TABLE 2.—*Effect of atmospheric and soil moisture on the seed setting of red clover, artificially cross-pollinated under controlled greenhouse conditions at Urbana, Ill., March, April, and May, 1927*

Plant letters and head numbers		Moisture		Average temperature	Times pollinated	Seeds set
Female parent	Male parent	Soil	Atmospheric, average relative humidity			
			Per cent	° F.	Number	Number
C-1	D-1	High	100	70	3	40
D-1	C-1	do	100	70	3	15
C-2	D-2	do	100	70	3	78
D-2	C-2	do	100	70	3	76
B-1	G-1	do	100	70	1	27
G-1	B-1	do	100	70	1	9
G-2	Selfed	do	100	70	1	0
B-5	do	do	100	70	1	0
C-3	D-3	do	55	70	2	42
D-3	C-3	do	55	70	2	23
E-1	C-5	do	55	70	1	9
C-5	E-1	do	55	70	1	13
C-4	Selfed	do	55	70	2	0
D-4	do	do	55	70	2	0
B-2	do	do	55	70	1	0
G-3	do	do	55	70	1	0
B-4	L-2	Medium	100	78	2	16
L-2	B-4	do	100	78	2	28
J-2	A-1	do	100	78	1	30
J-3	A-1	do	100	78	1	36
A-1	J-2	do	100	78	1	62
H-4	C-6	do	72	75	2	28
C-6	H-4	do	72	75	2	33
B-3	H-1	do	55	70	1	26
H-1	B-3	do	55	70	1	37
L-3	H-3	do	55	70	1	56
H-3	L-3	do	55	70	1	16
I-1	I-1	do	55	65	2	15
I-2	H-2	do	55	65	2	33
C-7	C-7	do	55	68	1	57
I-3	I-4	Low	80	70	1	21
I-4	I-3	do	80	70	1	21

In considering the number of seeds set, as given in Tables 1 and 2, it must be borne in mind that under field conditions an average of 25 seeds per head is considered to assure a fair seed crop.

In these experiments seeds in excess of this number were produced under conditions of high and of low atmospheric humidity and this even when the plants were growing in wet soil and the heads were in a saturated atmosphere.

The results of these investigations show, therefore, that high and low soil moisture and high atmospheric moisture do not prevent the setting of red-clover seed. Although considerable variation exists in the number of seeds set, the difference in the number produced under the various environments can not be interpreted as resulting from any one specific factor.

As has been pointed out, it was not possible to record the number of florets pollinated, and it is probable that foreign pollen was not transferred to every stigma. These facts render a strict statistical interpretation of the results impossible. It nevertheless remains true that seed was set under all conditions and in most cases as freely as could be expected in open-pollinated flowers in the field. There appears to be no material variation in the number of seeds set by reason of difference in atmospheric and soil moisture. Although most of the foregoing tests were conducted under extreme conditions of moisture, it is believed that intermediate conditions would give similar results.

If the florets had been in bloom for some time before they were pollinated, some ovules might have disintegrated before the pollen tubes reached them. This may have occurred in the case of heads C-1 and D-1, shown in Table 2. C-1, the female parent, produced nearly three times as many seeds as D-1. These heads were pollinated at three different times, head D-1 being approximately three-fourths mature when first pollinated, while C-1 was less than one-half in bloom. When recording the number of seeds set, it was noted that the seeds in C-1 were uniformly distributed throughout the head, whereas those of D-1 were located in the upper portion of the head, resulting in the setting of a decidedly smaller number of seeds. It seems probable that the ovules in the older flowers of D-1 had disintegrated before the pollen tubes reached them.

STUDIES UNDER CONTROLLED SOIL-MOISTURE CONDITIONS

SOIL MOISTURE AS A FACTOR IN GROWTH OF RED CLOVER

The quantity of seed produced by a given field of red clover will depend upon the number of flower heads, the number of florets, the number of florets pollinated, and the proportion of those pollinated that set seed. Soil moisture may affect any or all of these factors except the number pollinated. Under natural conditions this will depend, of course, upon the number and activity of the pollinating insects. The number of flower heads and the number of florets produced may be considered as an expression of the vigor of the plant as influenced by the moisture content of the soil. This may or may not have any relation to the effect of soil moisture on the effectiveness of pollen when applied to the stigma.

In the present study consideration was given to all of these characters, viz: The number of flowering stems and their height, as express-

ing vigor of growth; the number of flowering heads and the number of florets per head, as indicative of potentialities for seed production; and the number of florets that produced seed after being artificially pollinated, as showing the effect of various soil-moisture conditions on the receptivity of the stigmas.

During January, 1927, 27 galvanized-iron cans, 30 inches deep and 12 inches in diameter, painted with acid-proof enamel, were each filled with 135 pounds of the surface soil of Muscatine silt loam, uniformly compacted. Six additional cans were similarly filled with dune sand. Triplicate moisture samples of each soil type were taken as the cans were being filled, the loam containing 30.4 per cent and the sand 4.4 per cent moisture. The water-holding capacity of the



FIGURE 2.—Shelter used in maintaining soil moisture. Urbana, Ill., 1927

loam, as determined by the Hilgard method (2), was 85 per cent of the dry weight, and that for the sand was 33 per cent.

In order to avoid wide variations in seed production due to the genetic constitution of the plants, the seed used in these experiments was harvested in 1926, from two vigorous red-clover plants. The seeds were sown during the latter part of February, 1927, and the cultures were placed in the greenhouse where an approximate temperature of 60° F. was maintained. The same quantity of water, which was slightly below that needed for rapid growth, was applied to each can. On April 1, the plants were thinned to five per can, and later they were gradually hardened off. During the latter part of May they were placed under an especially constructed shelter, shown in Figure 2. In order more nearly to approximate natural conditions, the shelter was roofed with "Cel-o-Glass," which permitted the entrance of various wave lengths of the spectrum. In an effort to maintain more uniform soil temperatures, the cans were placed in sections of embedded draintiles.

On June 1, the moisture content of the soil in the cans was altered to low, medium, and high. While the capillary capacity as determined by the Hilgard method was not accurate, an approximation was secured as a guide by which the changes in soil moisture of each soil type could be made, as follows: High soil moisture, represented by the capillary capacity; medium soil moisture, 60 per cent of the water-holding capacity; and low soil moisture, 25 per cent of the water-holding capacity. After sufficient time had elapsed for moisture in the soil to approach a state of equilibrium, those cultures representing a high soil-moisture condition were supersaturated. The quantity of moisture applied to the soil of the other two sets appeared to be satisfactory. The quantity of moisture applied to the high-moisture series was therefore arbitrarily lowered until the water applied covered the surface of the soil approximately to a depth of one-fourth inch. A sufficient number of cans, filled with Muscatine silt loam were available for eight replications of each soil-moisture condition during the first growth of the plants. Duplicate tests of low, medium, and high soil moisture were conducted with cans filled with dune sand.

The cans were weighed two or three times a week, depending upon the prevailing environmental conditions, and the necessary water to correct for the quantity lost was added to the soil surface by spraying. The plants of the first growth were harvested individually August 3, 4, and 5, being cut $1\frac{1}{2}$ inches above the surface of the soil. Records were taken of each flowering stem as to its height, the number of flower heads and their maturity, the size of leaf and head, the color of leaf, and the vigor of the plant. Moisture-free weights were determined for each plant.

INFLUENCE OF SOIL MOISTURE ON DEVELOPMENT OF RED-CLOVER PLANTS

The foliage of the plants in soil of high moisture content became light green in color soon after they were subjected to the high moisture condition. This coloration of the leaves gradually increased; many became brown and others yellowish green by the time the plants were harvested. The leaves were also decidedly few and small when compared with leaves of plants grown under conditions of medium soil moisture. Adventitious roots developed from the lowest nodes of many flowering stems. The plants were markedly stunted, and the growth was lacking in vigor. The flower heads began blooming more than a week earlier than those on plants growing in soil of medium moisture.

Plants growing in soil of medium moisture developed vigorously, producing many flowering stems, medium-sized leaves normal in color, and large flower heads. The blooming of the flower heads was later than that of plants growing in soil kept at a high or a low soil-moisture content.

Plants growing in soil low in moisture were stunted, the leaves were of average size, but the heads appeared to be smaller than those from plants growing under a medium soil-moisture condition. Three weeks after the moisture content in this soil was lowered, the leaves showed symptoms of wilting during the day. This condition was more pronounced in the lower leaves, many of which turned brown. When the soil became dry enough to cause wilting, the green color of the plants became darker than normal.

Plants growing in dune sand maintained at soil moistures comparable to those used in the loam, developed similar external characteristics, as illustrated in Figure 3. The number of flower stems, number of heads, height of stems, and extent of vegetative growth were found to be adversely affected by extremely low or high soil moisture, as shown by Table 3.

Significant increases of the measured characters resulted from medium soil moisture when compared with low and high soil moisture.



FIGURE 3.—Effect of soil moisture on the development of red-clover plants grown in Muscatine silt loam (A) and in dune sand (B) at Urbana, Ill., 1927

No significant differences were observed in the effects of low and high soil moisture on the production of flower stems and the average height of the plants.

TABLE 3.—Effect of low, medium, and high soil moisture on the production of flower stems and heads, and weight of dry material of red-clover plants grown in Muscatine silt loam and dune sand at Urbana, Ill., 1927

Soil type and soil-moisture condition	Plants	First growth, average per plant of—			
		Flower stems	Height of stems	Heads	Weight of dry material
Muscatine silt loam:	Number	Number	Inches	Number	Grams
Low.....	45	4.7±0.20	10.1±0.37	11.5±0.83	5.45±0.227
Medium.....	45	7.5±.27	17.9±.41	30.5±2.08	13.60±.578
High.....	43	3.8±.17	11.9±.56	4.0±.38	3.21±.188
Dune sand:					
Low.....	9	5.7±.51	9.7±.54	11.3±1.80	4.99±.509
Medium.....	10	7.2±.25	17.0±.44	29.2±3.21	12.41±.828
High.....	10	3.2±.37	11.7±1.10	3.7±.54	2.60±.371

* Based on 9 plants.

Even though the effect of low and high soil moisture was much the same on all these characters, yet in general a low soil-moisture content was less injurious than a high one. The effect of soil moisture was the same whether the plants were growing in Muscatine silt loam or dune sand, except that in dune sand there was no significant difference in the number of flower stems in plants growing in soil of low moisture as compared to soil of medium moisture, but plants growing in dune sand kept at a high moisture content produced fewer flowering stems. This lack of difference between plants in soil of low and soil of medium moisture content probably resulted from the fact that one plant was missing from one of the low-moisture cans, giving the other four more available water for development. Dune sand under the existing condi-

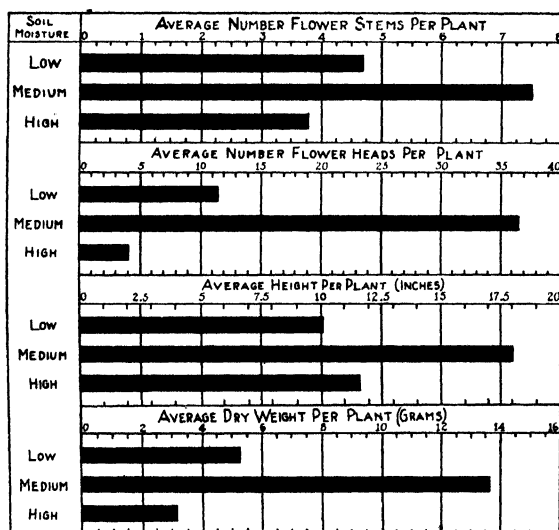


FIGURE 4.—Effect of soil moisture on the growth of red-clover plants in Muscatine silt loam, Urbana, Ill., 1927. (Based on Table 3)

tions was nearly as productive as the Muscatine silt loam, as is shown in Figures 4 and 5. The maximum productivity of either soil, however, was probably not reached. It is apparent that plants growing under optimum soil-moisture conditions will produce a greater yield of seed than those growing under more adverse conditions, provided the other factors that influence the setting of the seed are similar.

INFLUENCE OF SOIL MOISTURE ON NUMBERS OF FLORETS PER HEAD

Plants growing in loam or in sand, low or high in moisture, appeared to develop smaller heads than plants growing in soil of medium moisture content. In order to test the correctness of this observation, the number of florets per head was determined for five mature heads from each of two different mother plants growing in the same can. A statistically significant difference in the number of florets per head was noted in favor of heads produced by plants growing in loam soil, low or medium in moisture content in comparison with those produced by plants growing in saturated soil. (Table 4.) Although the figures show a difference in the number of florets between plants

growing in soil of low moisture as compared to those in soil of medium moisture, calculated odds indicate that the difference is not significant. In dune sand a significant difference in number of florets per head occurred only between plants in soil of medium and those in soil of high moisture. Since the heads appeared smaller on plants in soil low in moisture, this was probably due to a lack of turgor in the cells of the corolla tissue, which permitted the individual florets to remain close together. A wide variation in the number of florets per head occurred under each condition of soil moisture. Greater variability in the

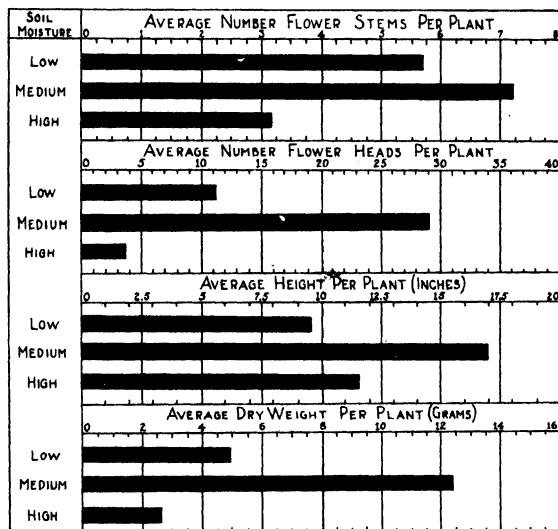


FIGURE 5.—Effect of soil moisture on the growth of red-clover plants in dune sand, Urbana, Ill., 1927 (Based on Table 3)

size of head occurred in both loam and sand when the plants were grown under extreme moisture conditions than when they were grown in soil of medium moisture.

TABLE 4.—Effect of low, medium, and high soil moisture on the number of florets per head for five mature heads from each of two mother plants when red-clover plants were grown in Muscatine silt loam and dune sand at Urbana, Ill., 1927

Soil type and soil-moisture condition	First growth of—			
	Flower heads	Total florets	Florets per head	Variability W^a
Muscatine silt loam:				
Low	80	7,209	90±1.7	4.42
Medium	90	8,512	95±1.8	4.33
High	77	6,208	81±2.3	4.81
Dune sand:				
Low	20	1,706	85±3.4	4.52
Medium	20	1,952	98±2.5	3.94
High	19	1,406	74±4.9	5.50

^a Weinberg's (7) formula of variability: $W = \sigma \frac{\sqrt{Mm - Mo}}{\sqrt{(Ma - Mo)(Mm - Ma)}}$

Where

σ = standard deviation.
 Mm = highest class interval or highest variate.
 Ma = mean.
 Mo = lowest class interval or lowest variate.

INFLUENCE OF SOIL MOISTURE ON MATURITY OF FLOWER HEADS

When the first growth was harvested records were kept on the maturity of all flower heads, each head being classified as brown, red, or "bud." The heads were classed as brown when all the corolla tubes were brown; red, when any open floret showing color remained on the head; and bud, when the head was without any opened florets. Heads from plants growing in soil low and high in moisture matured more uniformly than those growing under favorable soil-moisture conditions. (Table 5.) This was true whether plants were grown in dune sand or in Muscatine silt loam.

TABLE 5.—*Effect of low, medium, and high soil moisture on the maturity of flower heads of red-clover plants when grown in Muscatine silt loam and dune sand at Urbana, Ill., 1927*

Soil type and soil-moisture condition	Number of plants	First-growth maturity of flower heads					
		Brown		Red		Bud	
		Number	Per cent	Number	Per cent	Number	Per cent
Muscatine silt loam:							
Low.....	45	377	72.8	92	17.8	49	9.4
Medium.....	45	1,067	64.4	274	16.7	311	18.9
High.....	43	149	87.1	9	5.3	13	7.6
Dune sand:							
Low.....	9	80	78.4	13	12.8	9	8.8
Medium.....	10	182	62.3	63	21.6	47	16.1
High.....	10	32	86.5	2	5.4	3	8.1

INFLUENCE OF SOIL MOISTURE ON FERTILIZATION OF RED-CLOVER FLOWERS

It has been shown that a low or high soil-moisture content adversely affects the number of flowering stems, the number of heads, and the weight of dry matter produced by red-clover plants as compared to plants growing in soil of medium moisture. The difference in the number of florets produced under the various degrees of soil moisture was found to be slightly in favor of medium and low soil-moisture content. It remained to be determined whether under the conditions of the experiment there would be a difference in the number of seeds set by plants growing in soil of low, medium, or high moisture content. In the greenhouse experiments reported earlier in this paper no such difference was found.

It was hoped that plants growing in the open, like those under experiment, would be adequately pollinated by bees. From preliminary experiments conducted in 1926 it was learned that natural cross-pollination by bumblebees could not be depended upon for quantitative results in the matter of seed produced, even when colonies of bumblebees were transferred to locations adjacent to the controlled cultures.

Artificial cross-pollination, therefore, had to be resorted to, and florets were cross-pollinated between June 30 and July 20 from each of two individual plants of a different parentage growing in the same can. On most of the heads the number of florets successfully manipulated were counted, but in a few cases this was impossible on account of the condition of the florets.

From the number of seeds set, as shown in Table 6, it appears that high soil moisture is less favorable for seed setting than medium and low. It is believed, however, that the lower percentage of seed produced under high soil moisture resulted from a lack of pollen necessary for pollination rather than from a failure of the pollen or stigmatic surfaces to function. This deficiency of pollen was apparent in every case where plants were growing in soil of high moisture content. It may be assumed that the lack of pollen resulted from the unthrifty condition of the plants. Examination of the pollen from plants growing under the three moisture conditions failed to show any distinct differences in its physical characters.

TABLE 6.—*Effect of low, medium, and high soil moisture on the number of seeds set by flowers, artificially cross-pollinated, when two plants were growing in the same can in Muscatine silt loam and in dune sand at Urbana, Ill., 1927*

Soil type and soil-moisture condition	First growth				
	Number of plants	Total florets on head	Number of florets cross-pollinated	Florets setting seed	
				Number	Per cent
Muscatine silt loam:					
Low	10	968	385	263	68.3
Medium	12	1,204	468	369	78.8
High	14	1,106	475	281	59.1
Dune sand:					
Low	4	455	150	121	80.7
Medium	4	428	160	101	63.1
High	4	176	* 143	73	51.0

* Number of cross-pollinated florets on 2 heads approximated.

FIELD STUDIES

RESULTS OF POLLINATIONS MADE IN JUNE, JULY, AND AUGUST, 1927

In the previous investigations where plants were grown under controlled conditions, the degree of soil moisture and atmospheric humidity did not limit the setting of red-clover seed. In the field, many influences are operative, and it is difficult to separate and control any one factor. It was desirable, therefore, to determine how far the conclusion reached by a study of red-clover plants under control would be confirmed by a study of plants in the field.

The material available for this experiment consisted of vigorous second-year clover, growing on Muscatine silt loam in a high state of fertility. It was decided to cross-pollinate florets during the blooming season, when the combined influence of various atmospheric and soil-moisture conditions might be met.

Two red-clover plants were selected a foot apart, and 12 soil borings were made in a clockwise manner in a radius of 1 foot from the nearer plant. Moisture determinations were made from each boring of each of three zone depths, 9, 18, and 28 inches. The results of these determinations indicated that four equidistant borings represented the soil moisture of the main soil area from which water was absorbed by the roots. The borings were made, generally once or twice a week, from June 4 to September 22, and the percentage of moisture of each sample was determined. Each plant to be cross-pollinated was used as an axis from which the borings were located.

In order to obtain records of atmospheric moisture at the time the pollinations were made, a standard United States Weather Bureau instrument shelter was constructed and located at one edge of the plot. The shelter was so situated that when a hygrothermograph was placed inside, the instrument was 18 inches from the surface of the soil, a distance approximating the height of the clover heads. A sling psychrometer was used daily to check the readings of the hygrothermograph at the time of pollination.

Practically every day during the months of June, July, and August and during the first three weeks of September two plants located not more than a foot apart were selected for experimentation. Each plant had two flower heads of approximately the same degree of maturity, that is, from one-fourth to one-half in bloom. The heads were tagged and numbered, and one head from each plant was pollinated with pollen from a head on the other plant. The manipulation was similar to that used on plants in the greenhouse experiments. The greater number of the pollinated florets were manipulated three times, an effort being made to cross-pollinate all the florets on each head. The different pollinations of the same heads, however, were usually made two days apart. Pollinations were made during various hours of the day from 8 a. m. until 7 p. m. When the heads were cross-pollinated, observations were recorded as to whether the day was cloudy, partly cloudy, or clear; the time and date of the manipulation; the maturity of the heads; the physical condition of the pollen; and other notes descriptive of environmental and plant conditions. The heads were harvested four to five weeks after they had been pollinated, and notes were recorded as to the height, number of flower stems and heads, vigor, and the presence or absence of seed on open-pollinated heads of the female plant.

Because of the increase of insects which destroyed many florets and heads during the latter part of August it became necessary to bag the heads after pollination. Under these circumstances only one head with approximately 30 open florets on each plant was selected and cross-pollinated. The florets were counted and the heads covered with small cheesecloth bags until harvested. The total numbers of florets and seeds in each head were later counted in the laboratory. The hygrothermograph records were employed as a basis for the calculation of the saturation deficit, which permitted comparisons to be made of the variations of the atmospheric moisture under varying temperatures.

Weather conditions were ideal for the tests, as extreme and normal relationships prevailed at different times during the season. The plots were on low ground, and during periods of heavy rainfall water stood for several days 3 to 4 inches over the surface of the soil in the lower areas.

During the season a total of 145 heads were cross-pollinated, including heads from both first and second growth. In most cases the florets on each head were pollinated at different times, usually under a different set of environmental conditions. The uneven opening of the florets necessitated more than one pollination.

The results of the pollinations made during June, July, and August clearly indicate that moisture is not a limiting factor in the setting of red-clover seed. In many cases where the combined effect of high atmospheric and soil-moisture conditions were operative at the same

time seed set as freely as when soil and air were relatively dry. At different times during this period florets from 11 heads were pollinated when the soil was saturated or supersaturated. In all of these cases the number of seeds set was as great as under conditions of lower soil moisture. Florets pollinated when they were wet with dew or rain produced seed. The number of seeds, however, was less than from heads under dry conditions. The washing of the pollen from the needle was believed to be partly responsible for this decrease of seed setting. Likewise, the degree of atmospheric humidity did not appear to affect the setting of seed. There appeared to be no difference in the relative quantities of seed set by florets of vigorous plants and by those of weak plants or between first-growth or second-growth flowers.

RESULTS OF HAND POLLINATION, SEPTEMBER, 1927

The results of the pollinations made during September are considered representative of those of the previous months, since similar climatic conditions prevailed. A presentation of the data is possible, as a known number of florets on each head were pollinated at one time under a definite set of conditions, which necessarily must include both atmospheric and soil moisture. (Table 7.) There was one failure to obtain a setting of seed during September, and this failure appeared to result from nonfunctional pollen. The other plant involved in the cross, however, produced 20 seeds from 22 pollinated florets.

TABLE 7.—*Effect of soil moisture on the setting of seed when the florets of red-clover plants were artificially cross-pollinated under field conditions at Urbana, Ill., September, 1927*

Soil-moisture range	Second growth			
	Number of plants	Number of florets cross-pollinated	Florets setting seed	
			Number	Per cent
18.0 to 22.5 per cent.	12	337	231	69
22.6 to 27.5 per cent.	21	583	418	72
27.5 per cent to saturation	16	406	273	67
Supersaturation	4	94	50	53

INFLUENCE OF SOIL MOISTURE ON SEED SETTING

Florets of four plants were manipulated when the soil was supersaturated, many when the soil was nearly saturated, but the greatest number of pollinations were made when the soil moisture appeared to be most favorable for growth. Low conditions of soil moisture were experienced at only one period and this did not represent an extreme condition.

The percentage of seed set when the soil was supersaturated apparently is somewhat lower than that set under other soil-moisture conditions. This difference is not significant, it is believed, because wide variations existed in all ranges of soil moisture, and the small number of individuals in the supersaturated range undoubtedly contributed to the position it occupies. It happens that two of the four heads produced percentages of 72 and 87, which are as great as or greater than the percentage set at the other soil-moisture ranges. Also, each of the lower ranges of soil moisture have at least three individuals which set as low a percentage of seed as the other two plants under the supersaturated condition.

INFLUENCE OF METEORIC WATER ON POLLINATION AND FERTILIZATION OF RED-CLOVER FLORETS

During these investigations, florets of six plants were cross-pollinated when their perianths were wet with dew or rain, and if the number of seeds harvested is compared with the florets pollinated under dry conditions it is apparent that meteoric water does not limit the functioning of the pollen and the stigmatic surfaces of red-clover florets. Manipulation of the florets was difficult, as the pollen was often washed off the needle, and it was sometimes questionable whether pollen was successfully transferred.

The results presented in Table 8 show that florets wet with dew or rain at the time of pollination are capable of seed production. The percentage of florets setting seed was greater than the number normally set under field conditions. The chances of the pollen not being successfully transferred were great and possibly caused the wide variation in the number of seeds set.

TABLE 8.—*Effect of meteoric water on seed setting, when florets of red-clover plants were artificially cross-pollinated under field conditions while flowers were wet with dew or rain at Urbana, Ill., September, 1927*

Plant No.		Number of florets cross-pollinated	Florets setting seed	
Female	Male		Number	Per cent
100.....	101.....	21	7	33
101.....	100.....	22	20	91
102.....	103.....	26	9	35
103.....	102.....	19	10	53
120.....	121.....	26	19	73
121.....	120.....	27	18	67
Total or average.....		141	83	59

When the florets were tripped, the pollen was observed to be clumped together in large masses. It appeared to adhere to dry parts of the needle, but no affinity existed between the pollen and the moistened surfaces. Pollen was scarcely visible on the proboscides, legs, and body hairs of bumblebees which were caught while working the florets at the time the hand pollinations were made. During the season two heavy, precipitous rains occurred, followed by high temperatures. This condition appeared to cause a deterioration of the pollen which had been dehisced preceding the rain. A few hours later, however, pollen from newly opened florets functioned when used in cross-pollination.

ATMOSPHERIC HUMIDITY AS RELATED TO SEED SETTING

Atmospheric humidity during September varied widely. The results from pollinating florets under these conditions are presented in Table 9. During this period, the florets were pollinated under atmospheric conditions such that the saturation deficit varied from 0.109 to 0.654. In a few cases, rain was falling at the time of manipulation, the florets being shielded so that the rain did not touch the floret or needle.

TABLE 9.—*Effect of atmospheric humidity on the production of red-clover seed under field conditions, when florets were artificially cross-pollinated at Urbana, Ill., September, 1927*

Saturation deficit range	Number of plants	Number of florets cross-pollinated	Florets setting seed	
			Number	Per cent
0 to 0.199	8	219	131	60
0.200 to 0.299	18	497	352	71
0.300 to 0.399	10	279	188	67
0.400 to 0.499	2	51	32	63
0.500 to 0.599	9	228	172	75
0.600 to 0.699	6	146	97	66

The results of these investigations agreed with those conducted in the greenhouse in that atmospheric humidity was not a limiting factor in the production of red-clover seed. Although it might appear from the data in Table 9 that a low saturation deficit was not so favorable for the setting of seed as a drier atmosphere, most of the heads worked on at a deficit ranging from 0 to 0.199 were pollinated when the florets were wet with meteoric water, which increased the chances of unsuccessful pollination. The hygrothermograph records showed that during practically every night the atmospheric humidity increased approximately to saturation.

DISCUSSION

The foregoing results show that high atmospheric moisture in both field and greenhouse studies did not limit the setting of red-clover seed. If, as Martin found, artificial germination of pollen is delicately adjusted to a water supply, the stigmatic surface must be able to regulate the water which it furnished in accordance with the atmospheric moisture present. As the atmospheric humidity often varies quickly and widely, the regulation of the water produced by the stigmatic surface must be rapid, and it seems improbable that this is the case. In Martin's investigations on artificial germination it appears more likely that factors other than humidity were operative which were not studied. The atmospheric moisture surrounding the pollen grain at germination is believed to be of little consequence in its functioning. This conclusion is further substantiated by the results of the investigations on the effect of meteoric water on pollination and fertilization of the florets. Seed was produced in the field when red-clover florets wet with meteoric water were pollinated. In these experiments the stigma had no control over the quantity of water that came in contact with the germinating pollen.

Soil moisture whether high or low does not prevent seed setting, although plants grown constantly in wet soil may set a smaller percentage of seed than those in a dry or medium-wet soil. This appears to be due not to the influence of moisture on the functions of pollen or stigma, but to the fact that the plant, being in an unhealthy condition, fails to produce sufficient pollen. Medium soil moisture is conducive to vegetative growth and the production of flowers, which if properly pollinated would of course produce the largest yield of seed. The question arises, however, whether plants grown in soil in which the moisture is slightly below the optimum for growth

would not by maturing their flowers more uniformly, be more satisfactory for seed production. While infertile ovules may occur, they are apparently not caused by moisture conditions and are not present to a greater extent in the first than in the second growth.

Although these investigations indicate that variations in moisture conditions surrounding the plant at the time of reproduction do not limit seed setting, it is believed that moisture indirectly affects red-clover seed production in that it appears to influence the activities of pollinating insects. Microscopic examination of bumblebees after visiting flowers wet with meteoric water showed an absence of pollen on their body hairs and proboscides. Either the pollen did not adhere after being moistened or it was washed off the bees by excess water. During the seasons of 1926 and 1927 it was observed that more bees were engaged in visiting flower heads between noon and 5 p. m. than in the morning. Hygrothermograph records showed that generally the largest saturation deficit occurred at the time of the greatest activity of the bees.

Red-clover pollen is often considered as being dry when dehiscence from the anthers. Observations made under a wide range of environmental conditions indicate that pollen is always somewhat sticky and moist. Hence, red-clover plants may be classified as entomophilous. During periods of small saturation deficits pollen did not fly as freely as when a large deficit occurred. When the florets were wet with meteoric water, the pollen was generally found in relatively large sticky amorphous masses. In a few cases, however, pollen was discharged when the florets were tripped.

When a mature floret is tripped, the sudden release of the filaments and anthers from the carina discharges a part of the pollen. A large proportion remains on the anthers until the pollen comes in contact with outside agencies. When the floret first opens, the pollen is usually in lumpy, amorphous masses and very seldom is any discharged when the floret is tripped, but as the open flower advances in maturity a greater quantity is ejected. During these investigations observations have shown that, in visiting flower heads, the bumblebee has a preference for those in full bloom and those in which the corolla tissue has become brown. At this stage it is possible that many of the ovules have disintegrated and the quantity of viable pollen is not so abundant as in florets less advanced in maturity. Considerable variation in the quantity of pollen which is ejected exists between different florets of the same head. The greater part of this variation, no doubt, results from a difference in the maturity of the flowers. In several cases florets have been examined in which the pollen appeared never to have been functional.

SUMMARY

This study concerns the relation of atmospheric and soil moisture to seed production of red clover.

Soil moisture, whether low, medium, or high, did not prevent the setting of red-clover seed either when plants were grown in the field or under controlled conditions.

Medium soil-moisture content when compared to low or high soil-moisture content increased the productiveness of red-clover plants with respect to both the number of heads and flower stems and vege-

tative growth. Plants grown in soil low in moisture produced a greater number of heads and more vegetative growth than those grown in soil high in moisture.

Low soil moisture and high soil moisture were more conducive to early flowering and uniform maturity than a medium soil moisture.

A greater number of florets per head were produced when plants were grown in soils of medium and low moisture content than when they were grown in soils of high moisture content. No significant difference was observed in the number of florets per head on plants grown in soils of medium moisture and those grown in soils of low moisture content.

The differences in soil moisture did not appear to affect the physical condition of the pollen.

Atmospheric humidity did not affect the setting of red-clover seed under greenhouse or field conditions. Pollen under conditions of high atmospheric humidity showed a greater tendency to cohere than under conditions of low humidity.

When the plants were growing under field conditions, cross-pollinated red-clover florets wet with meteoric water produced seed.

In laboratory tests not all pollen grains burst immediately when placed in water.

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AERIAL CROWN GALL OF THE APPLE¹

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INTRODUCTION

Malformations of the apple variously termed "aerial crown galls," "stem tumors," and "burr knots" have been well known for many years. Knight (9)² in 1809 referred to them in connection with their use in propagating affected varieties by cuttings. Darwin (2, v. 2, p. 39-40), evidently referred to them when he wrote: "In Chiloe [an island off the coast of Chile] the inhabitants possess a marvelously short method of making an orchard. At the lower part of almost every branch, small, conical, brown, wrinkled points project; these are always ready to change into roots, as may sometimes be seen, where any mud has been accidentally splashed against the tree." He then described how branches from these trees are buried in the soil in order to form shoots. Garman (4) illustrated typical galls and referred to them as a knot disease, probably due to a fungus. Hedgecock (6) described these swellings as a form of crown gall, basing his assumption on the fact that they rooted and formed the typical woolly-knot type of crown gall when buried in the soil. He also specifically stated that cuttings from these malformations readily threw out roots when placed in moist sand or soil, and he called attention to the fact that these swellings "develop, internally, incipient adventitious roots, which * * * break forth, * * * forming a warty knot." Smith, Brown, and Townsend (15) also believed these malformations to be a form of crown gall. Despite the fact that the possibilities of vegetative propagation by use of these malformations were apparently well known, this method is believed never to have been put into actual use in the United States, probably because of the belief that it would not be practicable.

In the literature there is known to the writers only one reference³ in which isolation of the crown-gall organism (*Bacterium tumefaciens* Sm. and Town.) has been reported. Taubenhaus reported this isolation but failed to state whether he tested the validity of the organisms he isolated. This test is a necessary safeguard against wrong interpretations.

Brown (1), in reporting experiments that extended over a long period, stated her inability to isolate the crown-gall organism from typical stem tumors. She also noted that while plant pathologists and entomologists in general have referred to this type of overgrowth as a manifestation of crown gall, this assumption was based on rather

¹ Received for publication Feb. 2, 1929; issued August, 1929.

² Reference is made by number (italic) to "Literature cited," p. 261.

³ ADAMS, J. F. DISEASES OF FRUIT AND NUT CROPS IN THE UNITED STATES IN 1922. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul. Sup. 28: 308. 1923. [Mimeographed.]

inconclusive evidence. Swingle (16) in view of this statement and as a result of observations concluded that these aerial galls were merely varietal responses and urged the further consideration of propagating these affected varieties by means of cuttings from these galls. Melhus (10) and several other investigators apparently agreed with this diagnosis. Muncie and Shippy (11) also reported negative results in isolations from burr knots of apple, but noted that certain environmental conditions favored the production of burr knots.

Siegler (13) isolated, from malformations on apple roots, an organism which appeared identical with the apple strain of *Bacterium tumefaciens*. This organism was tested on apple shoots, as well as on certain other hosts, in order to determine its pathogenicity. It was noted that inoculations with this organism on apple shoots, growing under greenhouse conditions, resulted in malformations which resembled typical aerial galls.

EXPERIMENTS

The experiments reported in this paper were undertaken mainly for the purpose of testing the pathogenicity of certain bacterial organisms isolated from malformations on grafted apple trees. It was soon ascertained that the strain of the crown-gall organism that appeared identical with the daisy strain of Smith et al. (15) failed, with rare exceptions, to cause infection when inoculated on tender shoots of the apple, whereas a certain other strain, which may be called the apple strain, readily caused infection. This is the strain referred to by Siegler (13) as being probably identical with the apple strain of Smith et al. (15) and as being pathogenic on certain hosts, including the apple. It is thought that this is the same organism that Riker et al. (12) classed as one of their nonpathogenic strains.

Inoculations were made mostly by means of needle punctures, either single or grouped. Hypodermic needles and scalpels were also employed. The hypodermic needle was a standard 24-gauge make, which at the suggestion of M. B. Waite was slightly modified to prevent clogging. The inoculum was placed at various depths in the tissues. In some cases the hypodermic needle by accident was forced entirely through the tissues so that it projected from the opposite side. Control punctures were always made in the same manner with the same type of instrument either on the opposite side of the same twigs or on twigs which would correspond to the inoculated ones so far as growth characteristics were concerned. Inoculations were generally made on the growth of the current year, but they were also made on limbs up to 6 years of age and 1 inch in diameter.

In most of the experiments young seedlings grown from seeds of varieties susceptible to the aerial form of crown gall were used. The seeds were mostly from open-pollinated fruits of the Chenango and Buckskin varieties. Inoculations were also made on 10-year-old Early Ripe and Givens trees. The details of the inoculation experiments are listed in Table 1.

TABLE 1.—*Results of inoculating apple stems with apple and peach strains of crown gall*

[In the eighth column the numerator of the fraction indicates the number of shoots inoculated and the denominator the number of punctures]

Experiment	Host		Date of inoculation	Duration of experiment (days)	Source of inoculum	Method of inoculation	Shoots and punctures		Shoots infected		Punctures infected	
	Variety	Age					Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent
No. 62-G.....	Northern Spy seedling	4 months.	June 13, 1927	34	Apple strain (474-B-1)	Hypodermic needle punctures.	130	1	100	5	16.6	
Control G.....	do.	do.	do.	34	Sterile water	do.	130	0	0	0	0	
No. 66-L.....	do.	do.	do.	34	Apple strain (474-B-1) in water suspension.	do.	125	1	100	6	24	
Control L.....	do.	do.	do.	34	Sterile water	do.	125	0	0	0	0	
No. 64-A.....	do.	do.	Aug. 15, 1927	60	Apple strain (474-B-1)	Single needle punctures	230	2	100	7	23.3	
Control A.....	do.	do.	do.	60	Sterile needle	do.	230	0	0	0	0	
No. 64-B.....	do.	do.	do.	60	Apple strain (486-101)	do.	230	2	100	10	33.3	
Control B.....	do.	do.	do.	60	Sterile needle	do.	230	0	0	0	0	
No. 64-C.....	do.	do.	do.	60	Peach strain	do.	230	0	0	0	0	
Control C.....	do.	do.	do.	60	Sterile needle	do.	230	0	0	0	0	
No. 85-B.....	Early Ripe	10 years.	Nov. 11, 1927	300	Apple strain (455-9-A-9)	do.	125	1	100	2	8	
Control B.....	do.	do.	do.	300	Sterile needle	do.	250	0	0	0	0	
No. 85-D.....	do.	do.	do.	300	Apple strain (474-B-1)	do.	125	1	100	1	4	
Control D.....	do.	do.	do.	300	Sterile needle	do.	250	0	0	0	0	
No. 85-G.....	do.	do.	Nov. 2, 1927	300	Peach strain	do.	125	0	0	0	0	
Control G.....	do.	do.	do.	300	Sterile needle	do.	125	0	0	0	0	
No. 114-B.....	Chenango seedling	1 year	Feb. 24, 1928	211	Apple strain (486-101)	do.	5100	5	100	7	7	
Control B.....	do.	do.	do.	211	Sterile needle	do.	5100	0	0	0	0	
No. 114-C.....	do.	do.	do.	211	Apple strain (487-6)	do.	5100	3	60	3	3	
Control C.....	do.	do.	do.	211	Sterile needle	do.	5100	0	0	0	0	
No. 114-D.....	do.	do.	do.	211	Apple strain (510-2)	do.	5100	4	80	9	9	
Control D.....	do.	do.	do.	211	Sterile needle	do.	5100	0	0	0	0	
No. 114-E.....	do.	do.	do.	211	Peach strain	do.	5100	0	0	0	0	
Control E.....	do.	do.	do.	211	Sterile needle	do.	5100	0	0	0	0	
No. 116-A.....	do.	do.	Feb. 29, 1928	206	Apple strain (486-1)	do.	20300	14	70	25	8.3	
Control A.....	do.	do.	do.	206	Sterile needle	do.	20300	0	0	0	0	
No. 116-B.....	do.	do.	do.	206	Peach strain	do.	20300	1	5	1	.33	
Control B.....	do.	do.	do.	206	Sterile needle	do.	20300	0	0	0	0	
No. 120.....	do.	do.	Mar. 1, 1928	205	Apple strain (486 and 505)	do.	550	3	60	3	6	
Control.....	do.	do.	do.	205	Sterile needle	do.	550	0	0	0	0	
No. 138.....	Buckskin seedling	4 months.	May 7, 1928	149	Apple strain (486-101)	Very shallow single needle punctures.	660	1	16.6	1	1.6	
Control.....	do.	do.	do.	149	Sterile needle	do.	660	0	0	0	0	
No. 140.....	Chenango seedling	do.	May 9, 1928	136	Apple strain (486-101)	Grouped punctures of 5 made with fine needle.	16	1	100	4	66.6	
Control.....	do.	do.	do.	136	Sterile needle	do.	16	0	0	0	0	
No. 143.....	do.	do.	do.	136	Apple strain (486-101)	Single needle punctures	330	2	66.6	7	23.3	

TABLE 1.—Results of inoculating apple stems with apple and peach strains of crown gall—Continued

Experiment	Host		Date of inoculation	Duration of experiment (days)	Source of inoculum	Method of inoculation	Shoots and punctures	Shoots infected		Punctures infected	
	Variety	Age						Num-ber	Per-cent	Num-ber	Per-cent
Control No. 146-B	Chenango seedling	4 months	May 9, 1928	136	Sterile needle	Single needle punctures	3/30	0	0	0	0
	Early ripe	10 years	May 11, 1928	122	Apple strain (486-101) in water suspension	Hypodermic needle punctures	5/75	3	60	9	12
Control B	do	do	do	122	Sterile water	do	5/75	0	0	0	0
Control No. 146-D	do	do	do	122	Apple strain (486-101) in water suspension	do	5/75	5	100	11	15
Control D	do	do	do	122	Sterile water	do	5/75	0	0	0	0
Control No. 150	do	do	May 18, 1928	115	Apple strain (486-101)	Single needle punctures	5/50	2	40	2	4
Control No. 151	do	do	do	115	Sterile needle	do	5/50	0	0	0	0
	do	do	May 22, 1928	111	Apple strain (486-101) in water suspension	Hypodermic needle punctures	16/320	16	100	58	18
Control No. 153	do	do	do	111	Sterile water	do	9/180	0	0	0	0
	Givens	do	May 25, 1928	108	Apple strain (486-101) in water suspension	do	8/160	8	100	20	12.5
Control No. 159	do	do	do	108	Sterile water	do	5/100	0	0	0	0
	Early ripe	do	July 11, 1928	43	Apple strain (486-101)	1-year old shoot girdled at base with scalpel	1/1	1	100	1	100
Control No. 160	do	do	do	43	Sterile scalpel	do	1/1	0	0	0	0
	do	do	July 14, 1928	38	Apple strain (486-101) in water suspension	Hypodermic needle punctures in leaf axil under bud	7/164	6	86	21	13
Control No. 162	do	do	do	38	Sterile water	do	4/109	0	0	0	0
	do	do	July 16, 1928	56	Apple strain (486-101)	Smear over 1½-inch cut area on 2-inch limb and punctured in	1/1	1	100	1	100
Control No. 166	do	do	do	56	Sterile needle	Sterile needle punctures	1/1	0	0	0	0
	do	do	July 27, 1928	45	Apple strain (486-101)	Grouped needle punctures of 5	2/10	1	50	5	50
Control No. 170	do	do	do	45	Sterile needle	do	2/10	0	0	0	0
Control No. 171	do	do	do	45	Apple strain (486-101)	do	1/6	1	100	5	83.3
Control No. 173	do	do	do	45	Sterile needle	do	1/6	0	0	0	0
	do	do	Aug. 7, 1928	34	Apple strain (486-101)	1-year old shoots girdled at base with scalpel	10/10	4	40	4	40
Control	do	do	do	34	Sterile scalpel	do	10/10	0	0	0	0

In an examination of this list of experiments it will be seen that infections were obtained consistently, but that a comparatively small percentage of infection actually resulted, when every puncture is considered. There were very few cases in which an inoculated twig failed to become infected in at least one of the punctures. The reactions obtained on apple shoots of various ages resemble in gross morphological respects the typical naturally occurring burr knots. (Fig. 1, A, B.) In their incipency many are indistinguishable macroscopically from the small swellings usually found just above the bud and generally considered to be aphid injury. (Fig. 1, C.) The organism has been recovered from the areas designated by arrows in this figure. These small swellings may enlarge quite rapidly until they consist of a mass of roots projecting through the epidermis. (Fig. 1, D.) When the swellings have reached this stage it has rarely been possible to reisolate the organism from them. In no case have adventitious shoots appeared; in these experiments swellings made up of root primordia invariably have resulted from infection. In no case have the control punctures become infected, and they have never shown any reaction other than the slight disturbance of the tissues which normally would be expected. In cases where punctures have been relatively small the wounds have practically healed over, as shown in Figure 1, E, whereas many of the inoculated areas when not actually resulting in apparent infection still show a greater disturbance of the tissues as shown in Figure 1, F. A shoot in one of the experiments representing the highest percentage of infection is shown in Figure 2, A, with the control in Figure 2, B. It is seen that these are macroscopically indistinguishable from the naturally occurring galls. Among the infections which were secured by means of inoculations there were comparatively few cases where there appeared an outgrowth consisting of a single root rudiment as illustrated in Figure 3, A. Reisolations have been made from these infections.

On older wood, as a result of a single experiment, the chances of securing infection are apparently greater. Figure 2, C, shows swellings produced at five out of six points of inoculation by means of needle punctures in groups of five. The control punctures, made on a near-by branch, have healed over.

Rather striking examples of excessive root formation resulting from inoculations are shown in Figure 3, B, C. It is probable that the excessive root growth was favored by moisture conditions due to the closeness of these inoculated areas to the soil.

The experiments in which shoots of the current year were girdled at the base by a scalpel smeared with the inoculum proved interesting in that root primordia appeared on these girdled areas. This result may account for the presence of aerial galls on pruning wounds. Additional evidence on this point is furnished by an experiment in which the inoculum was placed on a freshly made pruning wound by means of a scalpel. One large swelling, one-eighth inch in diameter, resulted in the cortical region on the inoculated wound which was approximately one-half inch in diameter. This experiment has been repeated with like results. The control wound remained free of any abnormal growth. The root primordia which developed on the girdled twig in experiment No. 159 are shown in Figure 2, D, with the control girdled region completely healed as shown in Figure 2, E.

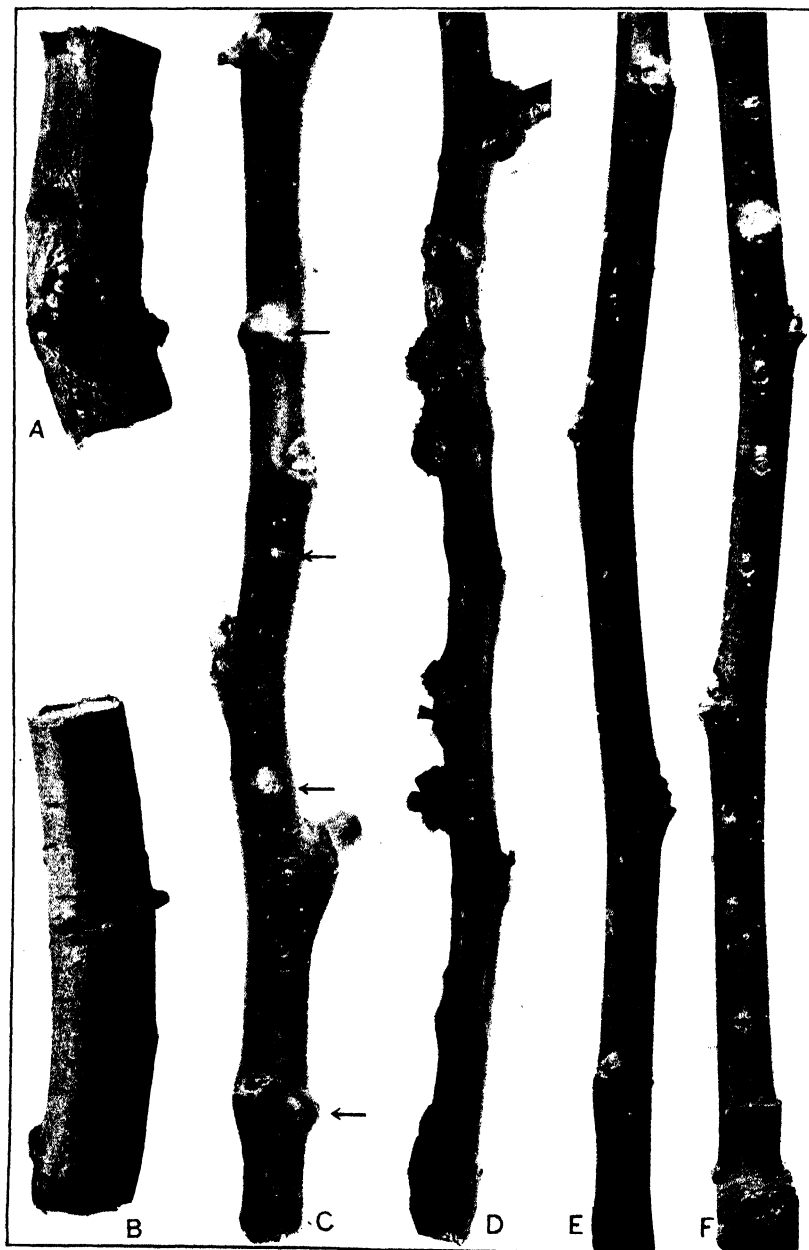


FIGURE 1.—A and B, Early stages of naturally occurring aerial galls on Early Harvest apple; C, smooth swellings which appear indistinguishable from woolly-aphis injury but which were induced by inoculation with the crown-gall organism on Early Ripe apple; time, 55 days; D, later stages of galls which were produced by artificial inoculations on a Northern Spy seedling and which show projecting roots; time, 400 days; E, control punctures on Early Ripe apple practically healed over; time, 155 days; F, shoot of Early Ripe apple on which only one puncture resulted in infection and on which the other punctures did not heal as they did in the control. All natural size



FIGURE 2.—A, Shoot showing a comparatively high percentage of galls resulting from artificial inoculations; time, 150 days; B, control punctures, which are healing over, for shoot shown in A; time, 150 days; C, infections from inoculations on older wood; time, 90 days; $\times 34$; D, root primordia formed in area girdled and inoculated by means of a scalpel in experiment No. 159; time, 43 days; E, control area healing after girdling with a scalpel; time, 43 days. All, Early Ripe variety. All except C natural size

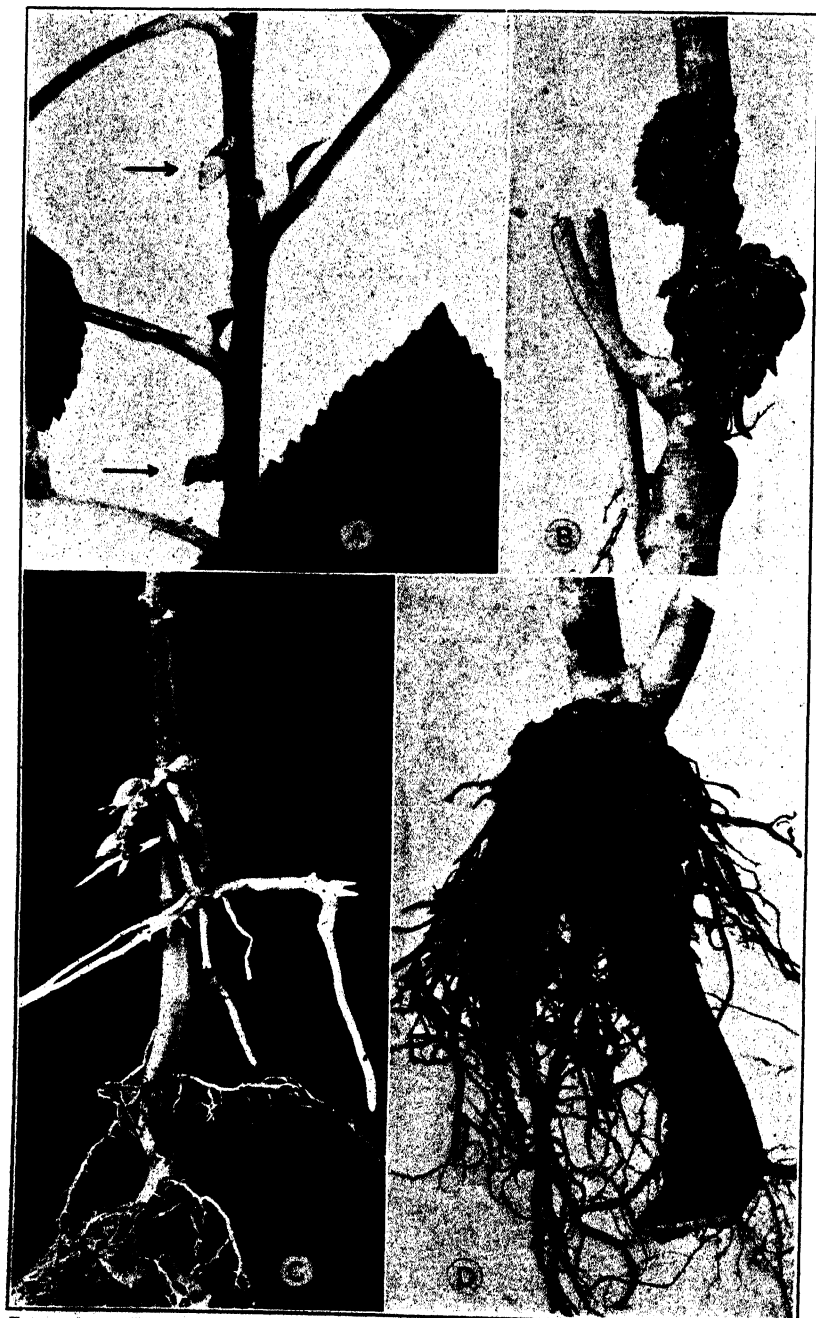


FIGURE 3.—A, Twig of a Northern Spy seedling showing single fleshy-root rudiments produced by artificial inoculations; time, 60 days; B and C, excessive fleshy root formation as a result of artificial inoculations on Chenango seedlings; time, 150 and 90 days, respectively; D, typical woolly-knot type of crown gall formed on Chenango seedling when aerial galls similar to those illustrated in Figure 1, D are planted in the soil; time, 206 days. All natural size

That these artificially produced aerial galls developed into the woolly-knot type of crown gall when planted in soil is shown in Figure 3, D. Numerous experiments repeatedly demonstrated this fact. The controls remained free of infection.

Attempts have been made to reisolate the organism from artificially produced galls of different ages. With but one exception it has been impossible to isolate the organism from galls over 65 days old. From galls less than 65 days old the organism has been reisolated only fairly consistently. In all there have been 16 attempts at reisolating the organism from artificially produced galls, and 31 per cent were successful. The reisolations have always been tested on susceptible hosts. During the last eight years numerous unsuccessful attempts have been made to isolate the crown-gall organism from naturally occurring aerial galls. In addition to these attempted isolations, macerations from galled areas have been made and the suspension inoculated into Bryophyllum. No infections were obtained on this host. All stages of natural galls have been used in these attempts at isolation.

DISCUSSION

Swingle (16) noted the absence in American literature of references to nonpathological, dormant, stem-borne roots in any plant. If this statement is applied to the apple and specifically to burr knots it is easily understood. Because of their resemblance to the underground form of crown gall, as stated previously, these malformations have been considered by pathologists as being of pathogenic origin. Previous to Swingle's (16) article, Brown (1) had reported her failure to obtain the crown-gall organism from the burr knots she described. Furthermore, before Hedgecock's (6) publication wherein he noted that these stem tumors developed incipient roots, the fact that these swellings consisted of root primordia had been well recognized. This recognition is true of Hedgecock's (6) statement that cuttings readily root, "developing directly into the woolly-knot form of the disease" (crown gall). It is not deemed necessary to enter into a further discussion to prove that aerial galls or burr knots have long since been recognized as root primordia and capable of readily propagating themselves by rooting when placed in the soil. That the terms "burr knot," "aerial galls," and "stem tumors" are synonymous must be taken for granted, since no one has ever explained how to differentiate them. Since it was generally accepted in this country that these aerial crown galls were of a pathogenic nature, pathologists may have been loath to suggest this means of propagation by cuttings.

From the writers' experiments it is evident that the so-called apple strain of *Bacterium tumefaciens* is capable of causing malformations morphologically identical with burr knots or stem tumors. Reisolations from relatively young infections are possible. The causal organism has been reisolated from one gall 106 days after inoculation, but the possibility that the original inoculum may have been present should not be overlooked. It has not been possible to culture the organism from older artificial infections or from naturally occurring galls of any age. It may be permissible to reason that the organism survives but a short time in the artificially produced galls. It may

also die very readily in the natural galls. Smith et al. (15), in discussing the loss of virulence in culture of these crown-gall organisms, raised the following questions: "Why should not virulence often disappear from organisms buried inside the tissues of tumors? And is not the fact that the tumor has ceased to be active and the host has gained the ascendancy evidence of this?" Israelsky (7) noted the disappearance of the organism from tumors and considered it due to the presence of a bacteriophage. Kauffman (8), using inoculum isolated from a mouse, also recorded failures to reisolate the organism from fully developed sunflower tumors, and concluded that after initial pathological growth has started the subsequent growth may be autonomous. This explanation may serve to account for the development of aerial galls after the initial stimulus has been supplied and definite root tissues have been differentiated. There is no normal way for these partially developed roots to function. They remain in a state of quiescence, although upon being placed in the soil under normal environmental conditions they become capable of functioning, at least to a certain extent. It is quite possible that the crown-gall organism may find this semidormant condition of the tissue uncongenial and the organism may die out because of this condition alone. Smith et al. (15), as noted above, adopted a similar line of reasoning when, in referring to the dying out of organisms and malformations produced by hyperplasia resulting from inoculations with the daisy strain, they noted the loss of virulence. The writers' viewpoint is that in these aerial tumors there is not a hyperplasia but an organization similar to a teratoma; that is, there are normal tissues occurring in a region where they are not normally found. They are apparently able to exist, but they may not offer conditions favorable to the growth of the organism. It should, therefore, be considered just as remarkable if the organisms lived in this tissue as if they either became attenuated or died.

That there are tissues in the stem of the apple capable of forming roots is generally recognized. The ease with which some varieties root from the scion part of the graft is evidence of this. Furthermore, certain varieties of pear and other fruits are propagated from cuttings which root quite readily under certain conditions. Just what stimulus is required for roots to be formed in an environment not normally favorable to their development is not known. The writers' experiments have proved that the introduction of the crown-gall organism into the stem tissues is at least one stimulus which causes the formation of root primordia. In regard to the failure to isolate the organism from naturally occurring galls there are many examples of other diseases from which the causal organism can not always be isolated. For example, the pear-blight bacillus is exceedingly easy to demonstrate in tissues at certain stages of infection, but most infections quickly die out.

The fact that burr knots are found more commonly on certain varieties does not necessarily make them a varietal characteristic as Swingle (16) argued. They may just as readily be regarded as evidences of varietal susceptibility. Nor does the occurrence of these tumors on vigorous trees prove or disprove their pathogenic nature. The writers' observations, however, are that, in general, mature trees which show numerous galls are rather weak. At the Arlington Experiment Farm the varieties chiefly affected are of no commercial

importance. While no attempt has been made to make a survey of this disease, it is known to occur generally throughout the world. In the writers' experience the Early Harvest appears to be the most important commercial variety on which galls are most frequently encountered. In 1918 at Jeff, Ala., every Early Harvest tree in an orchard of approximately 20 acres of Early Harvest and Yellow Transparent varieties was severely affected with burr knots. The trees were seriously weakened because the galls had almost girdled the main limbs and crotches. When one notes the great number of galls that may be present (fig. 4, A), and examines the areas surrounding the older burr knots, it is difficult to understand how they can be deemed uninjurious. The mechanical girdling effect in itself would appear to suffice, regardless of the means of entrance of decay organisms and the susceptibility of the affected areas to other injury as illustrated in Figure 4, B.

Hatton, Wormald, and Witt (5), after unsuccessful attempts to isolate an organism from burr knots, and as a result of other experiments, concluded that they are of a nonpathogenic nature. Doidge (3), however, noted the rapid spread of aerial galls on various hosts and evidently did not question the pathogenic nature of the galls. She noted that galls on the limbs and twigs usually originated at some point where an external injury had been caused by hail or other means.

The woolly apple aphid is capable of causing swellings on the roots of apples. These aphid galls are quite distinct from crown gall. This insect also spends part of its life on the woody tissues aboveground, and it is frequently found, in groups, in the regions of the leaf axils. Swellings form in those regions and elsewhere apparently as a result of the aphid punctures. Such swellings follow the feeding of aphid colonies on various parts of the tree. While there is no proof that aphids or other insects are agents in initiating and spreading aerial crown gall, observations and knowledge of the life history of this insect would suggest that this is quite possible. It seems probable, as Brown (1) suggested, that aphids could carry an inoculation into the punctures they make. It should be noted, however, that aphids have never been proved to be agents in transmitting the crown-gall organism to the roots. In the writers' experiments infection has seldom been secured by injecting the inoculum into the roots by means of needle punctures, while infection is more readily obtained on the shoots of the current year by inoculations with a needle of the same size. This difficulty in securing artificial infection in the roots by means of a puncture with a small needle may possibly account for the belief that aphids do not initiate and spread the crown-gall disease in the roots. A further discussion of the rôle they may play in connection with aerial crown gall is not within the scope of this paper.

That intumescences are produced on many hosts, including the apple, by means of chemical stimulation seems well established. Much experimental evidence is given by Smith, especially in his recent publication (14), wherein he concluded that "many of the common teratological forms in plants and animals are due to parasitic or mechanical traumatic displacements occurring in early life." Wallace (17) reported the production of intumescences on twigs on the Yellow Transparent variety by exposure to ethylene gas. These intumescences do not resemble aerial crown gall in that there is no



FIGURE 4.—A, Branches of an apple tree showing numerous galls; B, injurious effect of a gall to the area surrounding it. Both of the Rasmussen variety and greatly reduced

evidence of the root rudiments. This variety, however, is not considered susceptible to aerial crown gall.

The malformations obtained by Smith et al. (15) and by Brown (1) on apple shoots by inoculations with the peach strain of the crown-gall organism are quite distinct from the ones the writers have secured by means of inoculations with the apple strain. While root formations may be found in the galls illustrated by Smith et al. (15) and by Brown (1), it is evident that the swellings are a result of either hypertrophy or hyperplasia. The writers have rarely been able to secure infections on the apple by aboveground inoculations with the so-called peach or daisy strain, but this strain readily causes infection on the stems of peach and hosts other than the apple.

Smith et al. (15) noted differences in the strains of the crown-gall organism, but the fact that these differences have apparently not been generally recognized is probably the reason why inoculations similar to the ones described here have not been made previously.

SUMMARY

Malformations which are morphologically identical with those known as aerial crown galls, stem tumors, or burr knots have been produced consistently on the apple by means of inoculations with the apple strain of *Bacterium tumefaciens*.

The organism has been reisolated from these galls only within comparatively short periods after infection. It has not been isolated from naturally occurring aerial galls.

The evidence given here lends some support to the hypothesis that the natural occurrence of these malformations is due to infections with the apple strain of the crown-gall organism. It does not preclude the possibility that other agencies or factors may cause these malformations.

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SOME FACTORS INVOLVED IN THE WINTERKILLING OF ALFALFA¹

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INTRODUCTION

The importance of winterkilling in relation to agriculture is attested by the extensive literature on the subject which has accumulated over a period of years. Many types of freezing injury have been studied on a large number of different plants and from many points of view. Nevertheless, there still remains much to be learned before a comprehensive knowledge of all phases of this subject is obtained. It is the purpose of the present paper to present the data accumulated during the last two years in greenhouse and laboratory studies dealing with the freezing and killing points of alfalfa root tissue and some factors influencing them. It is recognized by the writer that some of the experiments, which have not been carried out on such a large scale or repeated so often as desirable, are inconclusive. The only reasons for presenting the results of such experiments at this time are the impracticability of continuing this work and the desire to make these data available to others interested in the subject.

As this paper deals with only a limited phase of winter injury no attempt has been made to give an exhaustive survey of the literature on this subject. Such reviews have been made by Chandler (4),³ Raos (18), Abbe (1), Wiegand (21), Blackman (3), Hildreth (9), Newton (16), Martin (14), and others. Steinmetz (19) has recently reviewed the literature on the overwintering of subterranean parts of herbaceous plants. The recent papers by Nelson (15), Leukel (12), Albert (2), and Graber, Nelson, Leukel, and Albert (6) dealing with the relation of root reserves to winterkilling of alfalfa are of interest here. Publications that have a special bearing on some particular phase of the work described in this paper will be mentioned in the discussion.

METHODS

Although most of the alfalfa plants employed in these experiments were of the Kansas Common variety, several other varieties and strains were also used. A strain of Kansas Common certified seed grown near Manhattan, Kans., was used throughout these experiments. The seed of the other varieties and strains was supplied by H. L. Westover, of the United States Department of Agriculture. The plants were grown in the field or in the greenhouse at Manhattan, Kans.

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² The writer is indebted to S. C. Salmon of the department of agronomy, Kansas State Agricultural College, for the use of his freezing chamber.

³ Reference is made by number (italic) to "Literature cited," p. 282.

The freezing point of alfalfa root tissue was determined by the thermoelectric method, a 2-junction thermocouple of the needle type having been used. The arrangement of apparatus was similar to that illustrated and described by Harvey (7). The readings were made with a Leeds & Northrup portable galvanometer. Sections of alfalfa root tissue about 2 cm. long were used. A hole was made in the tissue by means of a dissecting needle, and the needle of the thermocouple was inserted firmly in the opening. The tissue remained attached to the needle and was suspended in the freezing chamber in that manner. The ether surrounding the freezing chamber was kept at -9° to -10° C.

The killing point of alfalfa root tissue was determined by freezing the roots for different periods of time at different temperatures and under various conditions.

All the potted plants as well as part of those removed from the soil were frozen in the apparatus described by Hill and Salmon (10).

The remaining plants were removed from the soil, washed, and placed in a small sheet-iron chamber surrounded with ice and salt. After being frozen the plants were placed in soil in the greenhouse for observation. The potted plants, after being frozen, also were held in the greenhouse, and notes were taken frequently regarding their condition. In all cases unfrozen plants from the same lot were used as controls. Unless otherwise stated, at least 10 plants were included in each test, and several different periods of exposure were tried at each temperature in order to find the time required to kill the plants.

EXPERIMENTAL DATA

FREEZING POINTS OF ALFALFA ROOT TISSUE

Tests were made to determine the variations in the freezing points of alfalfa roots of different diameters, of sections of roots taken at the crown and at 10 cm. below it, and of different parts of the same cross section of a root.

In one experiment portions of root tissue from the crowns of 296 Kansas Common alfalfa plants of various ages (diameters) and collected at different times over a period of six months were frozen. The roots were gathered at about the same hour each time and while held were thoroughly wrapped in newspaper to reduce the loss of water. The thermocouple needle was inserted in the center of the cross section of the root to a depth of 8 to 10 mm. The results are summarized in Table 1.

TABLE 1.—Freezing points of alfalfa roots determined by the insertion of a thermocouple in the centers of cross sections taken near the crowns

Roots frozen	Diameter	Average freezing point	Roots frozen	Diameter	Average freezing point
Number	Milli-meters	$^{\circ}$ C	Number	Milli-meters	$^{\circ}$ C
1	1	-3.00	17	8	-2.14
13	2	-2.78	17	9	-2.09
31	3	-2.48	23	10	-2.26
42	4	-2.42	13	11	-2.12
57	5	-2.12			
43	6	-2.09	296	-----	-2.26
39	7	-2.09			

* Average.

These data show a low but statistically significant correlation ($r=0.215 \pm 0.038$) between the diameters of the roots at the crowns and the depressions of the freezing points. Regression, however, is probably nonlinear, as shown by an inspection of Table 1 and also as indicated by the comparative values of the correlation coefficient (r) and the correlation ratio (η). ($\eta=0.3568 \pm 0.051$ and 0.5131 ± 0.043 ; and $\zeta=0.2171 \pm 0.1504$ and 0.8265 ± 0.3000 .) The low correlation might have been due to the fact that the plants were of different ages and were growing under a variety of conditions with respect to moisture and temperature. To test this hypothesis, 42 plants of the same age, growing under the same conditions, were frozen. Two sections from each root were used, one from the crown and the other from 10 cm. below the crown. The diameters of these roots at the crown varied from 2 to 11 mm., and their freezing points ranged from -1.5° to -3.2° C. The correlation coefficient (r) for the sections taken at the crown was 0.094 ± 0.103 , and that for those 10 cm. lower was 0.037 ± 0.104 . This extremely low correlation may have been due, at least in part, to the small number of samples and to the fact that these random samples did not form a normal distribution curve. Most of the sections from the crown were 5 and 6 mm. in diameter, while a large part of the lower ones were 3 and 4 mm. thick. The remainder were distributed sparingly over a comparatively wide range. Eta was not calculated in these cases because of the small number of variables.

Another phase of this work was a comparison between the freezing points of the roots at the crowns and at 10 cm. below them. The 42 plants under consideration froze at average temperatures of -2.35° C. and -2.49° C. at the crowns and at 10 cm. below, respectively. The probable errors of these two samples were ± 0.025 and ± 0.035 , in the order mentioned. The difference between the averages of the freezing points was approximately 3.2 times the difference of their probable errors.

In order to obtain further evidence regarding the relation between the freezing points of the roots near the soil surface and at 10 cm. below, another experiment was conducted with 52 plants. The 52 samples taken at the crown froze at an average temperature of -2.34° C., and a like number from 10 cm. below, at -2.51° C. The freezing points of the sections from the two parts of the same roots varied from 0.1° to 0.3° C. in most cases, although occasionally both sections froze at the same temperature or that from the crown froze at a temperature slightly lower than the one 10 cm. below. The difference between the averages of the freezing points of the tissues of the two sets of samples was approximately four times the difference of their probable errors.

These data seem to show that there is a small but significant difference between the freezing points of the roots at the crown and 10 cm. below it; also that there is a small but statistically significant positive correlation between the diameters and the freezing points of the roots. The general trend of the two sets of data is similar and in the same direction; hence the difference in diameter may account at least in part for the variation in the freezing points of the roots at different levels.

An experiment was made to determine whether the tissue near the cambium froze at the same temperature as that at the center of the root. In this test two thermocouples and two galvanometers were

used. The thermocouple needles were placed in the same piece of root, one in the cambium or in the xylem near it and the other in the center. The tissue froze almost simultaneously throughout, as indicated by the two galvanometer readings. In only 3 out of 24 cases did the tissue of or near the cambium freeze at a lower temperature than that at the center of the root; in 17 it froze at a higher temperature; and in 4 both froze at the same temperature. The average freezing point of the tissue of or near the cambium in 24 roots was -2.11°C. , and that at the center of the root was -2.30°C. It should be stated that 20 of the roots used were 3 years old and the other 4 were 4 months old. However, approximately the same relative difference between the freezing points of the tissue at the center and that of or near the cambium was maintained in both cases.

As the freezing points of the tissues in different parts of the roots were found to vary slightly, in all subsequent experiments, except in cases where the roots were too small, the needle was placed in the xylem near the cambium. In a given experiment an effort was made to have the roots of as nearly the same diameter as possible.

FREEZING POINTS OF ROOTS OF DIFFERENT VARIETIES OF ALFALFA

Several experiments were conducted in which sections taken from the taproots near the crowns of different alfalfa varieties were frozen as in previous experiments. It was thought that perhaps some correlation would be found between the freezing points of the tissues and the observed hardiness of the varieties.

These experiments are summarized in Table 2. The figures represent the average of those obtained by freezing 5 to 10 plants. Young roots from the fields were frozen on November 12, when they were in a nonhardened condition, and again on February 11, when they were supposedly more hardened. The soil was frozen on February 11 and had been frozen for at least three days prior to that date. The air temperature for the same period varied from 30° to 4°F. (-1.1° to -15.6°C.) and averaged about 20°F. (-6.7°C.). Although the surface of the soil had been frozen slightly at times previous to November 12, it had usually thawed during the day, and there was no frost in the soil when the plants were dug. The air temperature, although cool at night, had been above freezing for several days except on the nights of November 8, 9, and 10, when it reached a minimum of 23°F. (-5°C.), 22°F. (-5.6°C.), and 26°F. (-3.3°C.), respectively. On the night of November 11 the minimum air temperature was 39°F. (3.9°C.).

The data given in Table 2 show that on November 12 the supposedly nonhardened plants, with the exception of the Hairy Peruvian, froze at lower temperatures than those frozen on February 11. The differences in several cases, however, are small and well within the limits of experimental error. These figures fail to show a correlation between the freezing point and the degree of hardening of the tissues. Furthermore, little or no correlation is shown between the freezing point of the tissue and the known winter hardiness of the varieties. Of those plants frozen on February 11, the Hairy Peruvian, which is perhaps the least hardy variety included, froze at a temperature lower than any of the others except Kansas Common; whereas Cossack, a hardy variety, froze at a higher temperature than any except Utah. There does seem to be some correlation between the

¹ "Variety" as used in this paper includes regional strains.

freezing points and the known hardiness of the varieties frozen on November 12; for example, Cossack, Kansas Common, and Grimm, which are all fairly hardy under Kansas conditions, froze at the lowest temperature, while the two Peruvian varieties, which are the least hardy, froze at the highest temperature. It is thought that this correlation may have been more or less accidental, as it did not hold consistently in other trials.

The average freezing points of 5-year-old roots from the same plots growing on the agronomy farm at Manhattan, Kans., on four different dates are also listed in Table 2. The figures for September 13-15 show a condition that occurred frequently, namely, that when roots were taken from extremely wet soil their freezing point was high. Under such conditions there was little undercooling and the exact freezing point was often difficult to ascertain. Many of these 5-year-old roots had been badly frozen during previous winters and were decaying, so that sometimes it was difficult to get consistent readings. This was especially true on February 3, when the work was discontinued although all the varieties were not then frozen. In these tests the thermocouple needle was always placed near the cambium. The figures in Table 2 show that on September 13-15, Kansas Common froze at a lower temperature than any other variety, while Italian, a nonhardy variety, ranked second; and, with the exception of Cape Lucerne and Argentine, Cossack, a hardy variety, froze at the highest temperature. Of the roots frozen September 19-21, 1926, Grimm, one of the hardiest varieties, froze at the highest temperature. Likewise, of the varieties frozen September 26-28, with the exception of Spanish, Grimm froze most easily. However, Grimm ranks as the hardiest among those varieties frozen on February 3, when the plants should have been in a hardened condition. Obviously, these data fail to show any consistent correlation between hardiness and the freezing point of the root tissue. This confirms the conclusions of Steinmetz (19), working with alfalfa, as well as those of other investigators working with other plants.

Many interesting data have been published by Wright and Harvey (24), Wright and Taylor (25, 26), Harvey and Wright (8), Diehl and Wright (5), Wright (22), Wright and Diehl (23), and others, showing that the freezing and killing points of various fruits and vegetables are practically the same.

The absence of any such correlation between hardiness and the freezing point of the alfalfa roots was further shown by an experiment conducted on February 10, 1927, in which only plants of the Kansas Common variety were used. There were four lots of plants, as follows: (1) 10-months-old plants brought from the field into the greenhouse on December 1, 1926; (2) plants of the same lot obtained from the field on February 10, 1927; (3) 5-months-old plants growing in large pots in the greenhouse; and (4) 5-months-old plants growing in large pots outside the greenhouse. The plants in lots 1 and 3 and in lots 2 and 4 should have been in a nonhardened and a hardened condition, respectively. The average freezing points of 10 plants of each lot were -3.36° , -3.1° , -2.59° , and -2.65° C. for lots 1, 2, 3, and 4, respectively. In this test the nonhardened plants in lot 1 froze at a lower temperature than the hardened ones in lot 2, whereas the nonhardened plants in lot 3 froze at a higher temperature than the hardened ones in lot 4.

TABLE 2.—Average freezing points ($^{\circ}$ C.) of the root tissues of different varieties of alfalfa

Date of freezing	Age of plants	Freezing points of varieties indicated															Condition of soil
		Cos-sack	Argen-tine	Hairy Peruvian	Smooth Peruvian	Kansas Common	South African	Utah	Grimm	Ladak	Spanish	Prov-ence	Turk-estan	Dakota 12	Cape Lul-cerne	Italian	
Nov. 12, 1926.	3 months.	-2.6	-2.35	-2.1	-2.25	-2.65	-2.4	-2.5	-2.85								Wet.
Feb. 11, 1927.	6 months.	-2.1		-2.5	-2.2	-2.6	-2.3	-2.1									Do.
Sept. 13-15, 1926.	5 years.	-1.5	-1.5			-2.37		-1.83	-1.72	-1.75	-1.65	-2.17	-1.6	-2.1	-1.0	-2.2	Very wet.
Sept. 19-21, 1926.	do.	-2.35	-2.2			-2.15		-2.62	-1.76	-2.96	-1.77	-2.0	-2.15	-2.15	-2.55	-2.5	Medium wet.
Sept. 26-28, 1926.	do.	-2.75	-2.78			-2.73		-2.81	-2.37	-2.71	-2.09	-3.1	-3.01	-2.72	-2.71	-2.77	Medium dry.
Feb. 3, 1927.	do.	-2.6				-2.17			-2.7	-2.5	-2.38						Wet.

THE KILLING POINTS OF ALFALFA ROOT TISSUE

The period of exposure which plants from the field will withstand varies considerably with their hardiness and with the conditions of the environment. For many years efforts have been made by investigators to develop a method that will enable them to acquire information regarding the conditions that influence the hardiness of plants and the effect of environment on the power of plants to withstand freezing. Most workers seem ultimately to reach the conclusion that some method of artificial freezing is the most satisfactory way to make such studies. The writer has frozen many alfalfa plants in order to learn something of the period of exposure necessary to kill them under different conditions.

PLANTS GROWING IN POTS

In these experiments some of the plants used were grown in pots in the greenhouse and the others were obtained from the field. The plants frozen on December 20, 1926, in the last experiment recorded in Table 3 had not grown in pots, but were brought from the field and set in soil in pots just before they were placed in the freezing chamber. In the next to the last experiment listed in Table 3, the plants were growing in 12-inch pots and, because of the insulating effect of the soil, were able to withstand a much longer exposure to a comparatively low temperature.

TABLE 3.—*Temperatures and exposures necessary to kill Kansas Common alfalfa plants of different ages*

[Plants exposed while growing in 8-inch pots except for those frozen December 12, 1926, which were growing in 12-inch pots]

Date of freezing	Source of plants	Age of plants	Probable state of hardening	Temperature		Period of exposure required to kill plants
				Average	Range	
		Months		°C.	°C	Hours
Feb. 16, 1927	Grown in field 7 months, and brought to greenhouse Dec. 1, 1926.	9	Nonhardened.	-20.5	-18 to -22	4½
Dec. 22, 1926	do.....	7½	do.....	-23	-21 to -26	4 to 5
Jan. 25, 1927	do.....	8	do.....	-9.2	-5 to -18	7½
Feb. 28, 1927	do.....	9	do.....	-6.5	-5 to -8	Undetermined.*
Dec. 10, 1926	Always grown in greenhouse....	14	do.....	-26.5	-23 to -30	3½
Dec. 20, 1926	do.....	14	do.....	-23	-21 to -26	3½
Jan. 26, 1927	do.....	15	do.....	-18.5	-5 to -21.4	½64
Jan. 25, 1927	do.....	15	do.....	-9.2	-5 to -18	7½
Dec. 12, 1926	do.....	8	do.....	-20.5	-18 to -22	24
Dec. 20, 1926	From field at time of freezing....	8	Hardened...	-23.5	-21 to -26	6

* Little injury in 8 hours.

The data presented in Table 3 show that under the conditions of these experiments nonhardened alfalfa roots growing in 8-inch pots were killed in from 3½ to 7½ hours at average temperatures varying from -26.5° to -9.2° C., respectively. Hardened plants from the field and nonhardened plants from the greenhouse, both frozen on December 20, 1926, withstood approximately the same average temperatures, namely, -23.5° and -23° C., for 6 and 3½ hours, respectively. The plants from the field showed considerably more resistance to cold than did those from the greenhouse; this can be explained

only by a variation in the degree of hardening. The nonhardened plants in 12-inch pots required freezing for 24 hours at an average temperature of -20.5° C. to kill them. Here the difference must be attributed to the larger bulk of soil about the roots.

PLANTS REMOVED FROM THE SOIL

Concomitant with the investigations discussed above, tests were made to determine the killing point of alfalfa roots when removed from the soil. Plants from the greenhouse and from the field were used. The former were nonhardened and the latter varied in degree of hardening. The degree of hardening of the field plants was estimated by studying the thermograph records of the temperatures covering a period of a few days prior to the time the plants were collected. When the temperature ranged from 0° to -20° C. for several days before the plants were frozen they were classed as hardened; when the temperature was above 0° C. for several days they were considered nonhardened. Slightly hardened plants were those collected after a cold period followed by warm weather for a day or two immediately preceding the day the plants were frozen.

The data presented in Table 4 show that the time necessary to kill small plants removed from the soil and frozen varies with the temperature, with the degree of hardening, and to some extent with the size of the roots. The plants grown in 12-inch pots out of doors until autumn and then in the greenhouse were killed in from 15 minutes to $3\frac{1}{2}$ hours at average temperatures of -5.5° to -20° C., depending upon the temperature. The plants which had grown in the field for seven months and in the greenhouse from December 1, 1926, to February 16, 1927, withstood an average temperature of -20° C. for 30 minutes, while the plants frozen on the same day and at the same temperature, recorded as the first line of Table 4, were killed in 15 minutes. The only difference that could be detected between these two lots of plants was in the diameters of their roots. The roots of the plants that withstood exposure for 30 minutes were twice as thick as those that were killed in 15 minutes. This difference was due to the fact that the former had grown in rows in the field for seven months, while the latter, although of about the same age, had grown in pots.

Plants taken from the field and considered to be hardened were frozen on December 22, 1926. These withstood an average temperature of -23.5° C. for three and one-half hours, which was a considerably longer time at a lower temperature than that endured by those of the same lot frozen on March 18 or February 16, 1927. March 18 and February 16 were preceded by two weeks and two days of warm weather, respectively, while December 22 followed a rather protracted cold period.

The effect of hardening is shown also in the results of freezing plants from seed sown broadcast in the autumn. These plants were fairly small, the taproots near the crowns measuring from 2 to 5 mm. in diameter. Two lots of these plants frozen on March 18 at temperatures of -17° and -18.5° C. were killed in 15 minutes. Another slightly hardened lot frozen on February 28 withstood an average temperature of -5° C. for five and one-fourth hours. On the other hand, the slightly hardened plants frozen on February 16 were killed in 30 minutes at -21° C.

TABLE 4. — *Temperatures and lengths of exposure necessary to kill young Kansas Common alfalfa plants of different degrees of hardening*

[Plants removed from the soil and placed in the freezing chamber at the recorded temperatures]

Date of freezing	Source of plants	Age of plants	Probable degree of hardening	Temperature		Period of exposure required to kill plants
				Average	Range	
Feb. 16, 1927	Grown in 12-inch pots out of doors until autumn, then moved into greenhouse.	Months 8	Nonhardened.	°C. -20	°C. -19 to -21	15 minutes.
Mar. 21, 1927	do	9	do	-18.6	-18.2 to -19	25 minutes.
Feb. 25, 1927	do	5	do	-15.6	-15.2 to -16	50 minutes.
Feb. 28, 1927	do	8	do	-5.5	-5 to -6	3½ hours.
Feb. 16, 1927	Grown in field seven months; transferred to greenhouse Dec. 1, 1926.	9	do	-20	-19 to -21	30 minutes.
Mar. 18, 1927	Grown from spring seedling; sown in rows in field.	11	do	-17		50 minutes.
Feb. 16, 1927	do	10	Slightly hardened.	-20.2	-19 to -21.5	1¼ hours.
Dec. 22, 1926	do	8	Hardened.	-23.5	-21 to -26	3½ hours.
Mar. 18, 1927	Grown from seed sown broadcast in autumn in field.	7	Nonhardened.	-17		15 minutes.
Do	do	7	do	-18.5		Do.
Feb. 16, 1927	do	6	Slightly hardened.	-21		30 minutes.
Feb. 28, 1927	do	6	do	-5	-4 to -8	5¼ hours.

The data presented in Tables 3 and 4 show that plants from the field varied in hardiness during the winter months. This variation in the time the plants endured a given temperature without being killed is correlated with the temperature to which they were exposed before they were frozen. Plants collected during a cold period were not so easily killed as those collected after a few warm days.

These data also show that the insulating power of the soil is an important factor in protecting the roots from freezing, since non-hardened plants removed from the soil before being frozen often were killed in 15 minutes, whereas they withstood the same temperature for three and one-half hours or longer when growing in soil in 8-inch pots.

A few tests were made during the winter of 1927-28 to determine the comparative hardiness of field and greenhouse plants. The plants were removed from the soil, washed, fixed between 12-inch pot labels in such a way that every other plant belonged to each lot being tested, and then placed top down in the freezing chamber cooled with ice and salt. The plants used were of the same age, but those from the greenhouse were larger, since they had continued to grow after those from the field had ceased to grow because of the low temperature. The field plants used in the experiments conducted on November 11 and January 10 were found to be no hardier than those from the greenhouse. However, on January 17 and 25 the former were less severely injured at a given temperature than the latter. On February 9 the difference in hardiness between the two sets of plants was very slight.

Less striking differences were obtained between the hardened and nonhardened plants in these experiments than in those recorded in Table 4. The differences probably would have been more pronounced, however, if the greenhouse plants had not been larger than those from the field.

FACTORS AFFECTING THE FREEZING OF ALFALFA ROOTS

DIAMETER OF ROOT

Certain factors other than the hardness of the plants must be taken into account in studying the freezing of alfalfa roots. For example, other things being equal, large roots usually require a longer time to freeze, as indicated by thermocouple tests, and also withstand a longer exposure at a given temperature without being killed, than do smaller roots. When not properly insulated, the small ends of the taproots of both hardened and nonhardened plants are killed first when subjected to experimental freezing. This seldom happens in the field, for there the cold can reach the roots only from above. Figure 1 shows two plants with the lower ends of the taproots killed by freezing. These plants grew in a 12-inch pot placed outside the greenhouse during the winter. A number of plants near the margin where they were not so well protected with soil showed this condition. Roots of alfalfa plants removed from the soil and then plunged into a freezing chamber often died from below upwards in this manner, so that plants whose crowns were apparently uninjured eventually died. There is another possible explanation of the freezing of alfalfa roots from below under certain conditions, namely, that the lower part of the root which is not subjected to such low temperatures during the hardening period is not so hardy as the upper part. In order to avoid this type of injury a freezing chamber which was open at the top was devised, and the plants were placed in it with the tops down. In this way the tops and upper parts of the taproots were exposed to the lowest temperature. This type of freezing chamber was used almost exclusively during 1927-28.

RATE OF THAWING

Still another factor which is thought by many to influence the killing of plants by freezing is the rate of thawing. No detailed study of this factor was made, but it was tested to some extent with respect to alfalfa by freezing young plants of the Kansas Common and Grimm varieties growing in pots at different temperatures and for different lengths of time. About 1,600 plants in all were used. One half of these were frozen and then thawed quickly in a warm greenhouse, while the other half were wrapped in felt, covered with sawdust, and kept in a cool basement for 24 hours or longer. Subsequently both lots were treated alike. No difference in the amount of killing was evident in any case. This does not prove that the rate of thawing is never a factor in winterkilling, but it does indicate that rapid thawing does not necessarily cause a greater amount of injury.

ROOT RESERVES

A correlation between the amount of root reserves and the longevity of alfalfa stands has been observed by a number of agronomists. This has received special attention in recent years in Wisconsin (2, 6, 12, 15) and in Minnesota (19). However, these workers do not seem actually to have demonstrated that alfalfa plants with low carbohydrate root reserves freeze more easily than similar plants with an abundance of such reserves. In order to obtain some definite information on this point the following experiment was conducted: Nine 8-inch pots in which were growing about ten 5-months-old

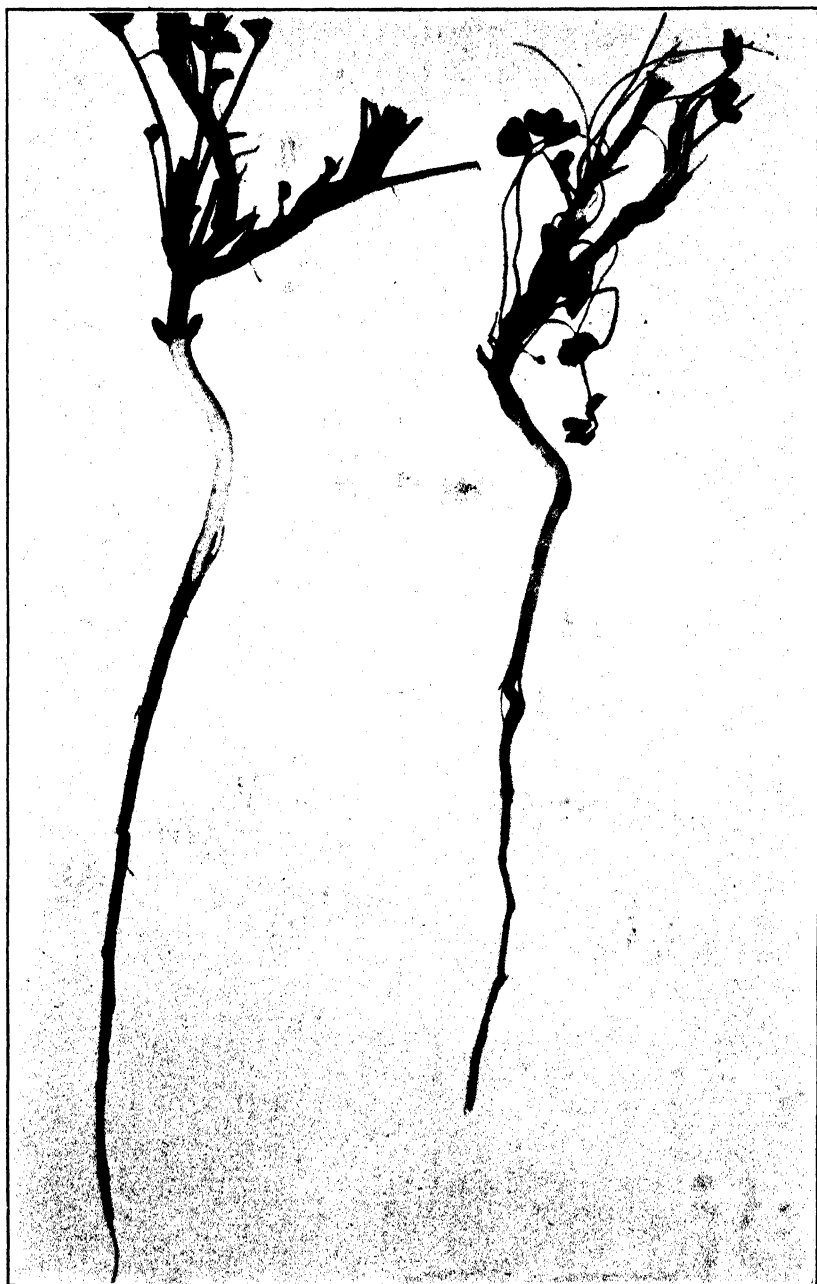


FIGURE 1.—Two alfalfa plants with the lower parts of their roots killed by freezing. They grew in a 12-inch pot outside the greenhouse during the winter. A number of plants near the margins of the pots where they were not well protected by soil were affected in this manner

Kansas Common alfalfa plants were held in the greenhouse in a box covered with heavy black threaded felt. This was shaded with newspaper to prevent the temperature from becoming too high. The plants were thus deprived of light but were provided with moisture and a temperature somewhat comparable, although probably slightly higher at times, than that to which the control plants were subjected. The control consisted of an equal number of pots from the same lot held near the box but left uncovered. The plants were placed in the box on January 24, 1928.

On January 31, when the first pots were removed from the box, the leaves had lost perhaps 75 per cent of their chlorophyll. The plants were removed from the pots, the tops cut off about 2 inches from the crowns, and the roots trimmed so as to leave taproots about 6 inches in length. These plants, together with those from the control pots treated in a similar manner, were placed tops down in the freezing chamber. Three lots of five plants each were frozen for 10, 15, and 20 minutes at temperatures of -5° to -6° C., -6° to -8° C., and -9° to -10° C., respectively, after which they were planted in a bed in the greenhouse. The plants frozen for 10 and 15 minutes were injured, but little or no difference was evident between those that had been deprived of light and the controls. However, plants frozen 20 minutes showed injury estimated at 75 per cent, as compared with 66 per cent for the controls.

In like manner plants from both sets of pots were frozen on February 9, and those that had been held in the dark were more severely injured than the controls. On this date the original top growth was about dead and new white shoots were being produced.

A third freezing was made on February 17, at which time the original tops of the plants held in the dark were dead and the new white shoots were 6 inches long. Plants of both lots were frozen for 15 minutes at temperatures ranging from -4.5° to -9° C. The control plants all lived, although two were weakened considerably, while all but two of the plants from the dark died. All plants of each lot treated in exactly the same way as the others except that they were not frozen grew well. A photograph of a pot from the box and a control pot taken March 3 is shown in Figure 2, A. The remaining plants were frozen on March 4, and those which had been deprived of light were much more severely injured than the controls.

This single experiment, although conducted on a very limited scale and with nonhardened plants, indicates that plants lacking root reserves are killed more easily by freezing than those with abundant root reserves. Judging from the data presented by other investigators (2, 6), the carbohydrate root reserves in the plants held in the dark in the writer's experiment probably were not entirely depleted even at the end of the experiment. However, a study of cross sections of some of the roots showed that the starch was entirely gone in plants deprived of light (fig. 2, C) and that it was very abundant in the control plants (fig. 2, B). No analyses were made to determine whether or not sugar was present in the former lot of plants. Since roots whose carbohydrate reserves were reduced by being deprived of light froze more easily than the controls, it seems probable that plants whose root reserves have been decreased by too frequent cuttings will likewise freeze more easily than plants with abundant root reserves.

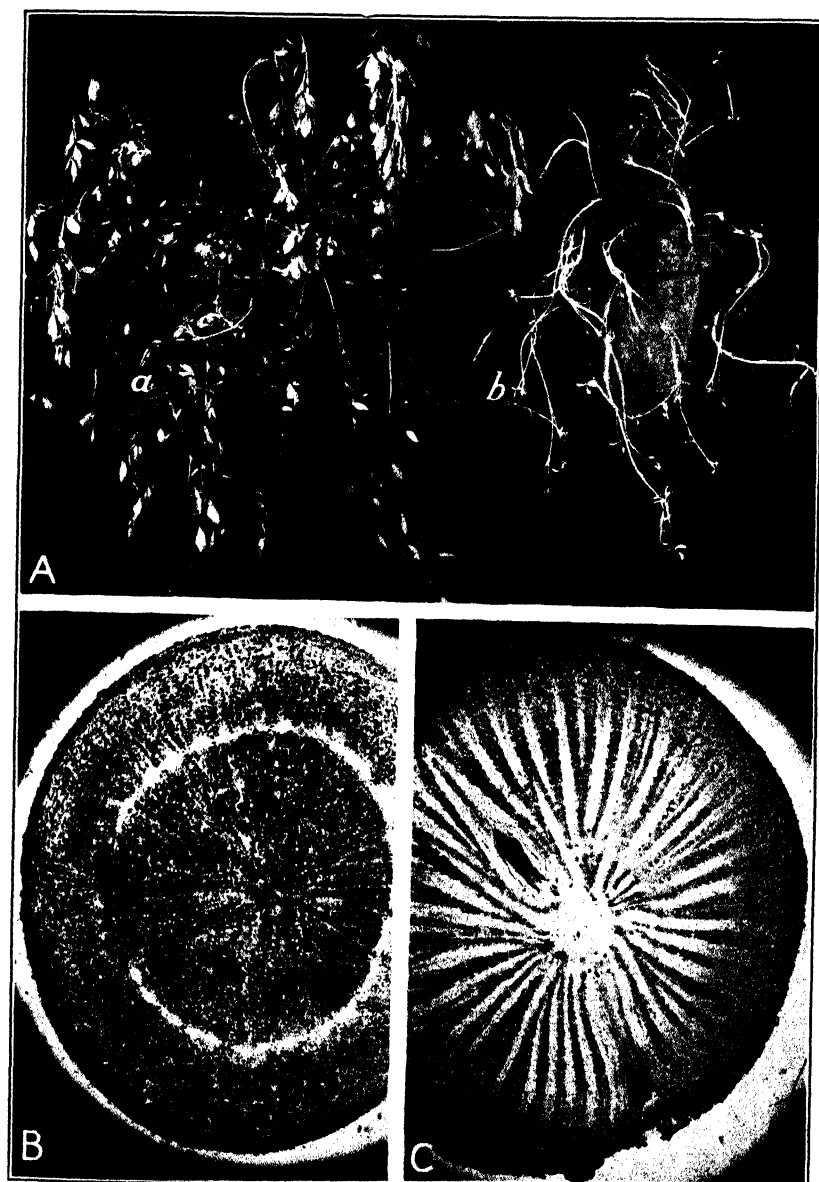


FIGURE 2.—A, Two pots of Kansas Common alfalfa plants of the same age. One pot (a) had grown continuously in the greenhouse, while the other (b) had been deprived of light by being inclosed in a box covered with black paper. The original tops of the plants in b died, and new white shoots were sent out. Such plants when transplanted to a bed in the greenhouse grew practically as well as those of the controls. B, Cross section of one of the roots in the control pot in A, a, showing the cells filled with starch as denoted by the black color. The starch was stained with iodine before being photographed. \times about 8. C, Cross section of one of the plants held in the dark box, showing the complete absence of starch. Plants deprived of light were more easily killed by freezing than were plants grown in the light. \times about 8.

ENVIRONMENTAL CONDITIONS

SOIL MOISTURE

The moisture content of the soil markedly influences the resistance of potted plants to freezing injury. Hill and Salmon (10) found that when wheat plants growing in dry soil were frozen under artificial conditions, they were injured much more severely than similar plants growing in wet soil. This difference they attributed to the high specific heat of water, which in a wet soil prevents a rapid change in temperature

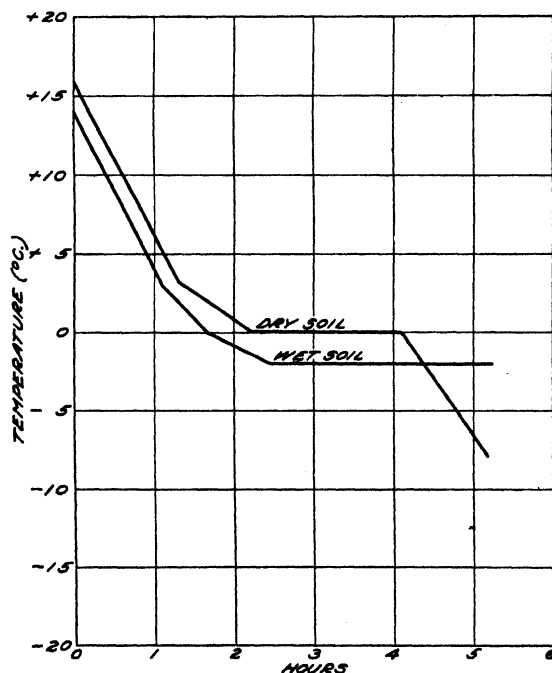


FIGURE 3.—Comparative rate of temperature fall in wet and dry soil contained in 8-inch pots and placed in a freezing chamber which had an original temperature near 0° C., but which was lowered rapidly to -10° C. and more slowly to -16° C.

with the result that plants in dry soil are exposed to a lower temperature than those in wet soil. The extent to which the moisture may influence the lowering of the temperature of the soil was illustrated in an experiment conducted by the writer in which two 8-inch pots, one with wet and the other with dry soil, were placed near each other in a freezing chamber. A thermometer was placed in the soil in the center of each pot, and frequent readings were made. The temperature of the wet soil dropped to 0° C. in 1½ hours. In another 50 minutes it reached -2° C., and it remained at that temperature throughout the experiment, or for about 2½ hours longer. On the other hand, the dry soil required 2½ hours to reach 0° C., where it remained for 1½ hours, and then dropped to -8° C. in 65 minutes. Hence the temperature in the wet soil reached -2° C. while that in the dry soil reached -8° C. in approximately 5½ hours. The complete data are presented graphically in Figure 3. These results simply show what happened in one instance; the effect of moisture would of course vary with the amount of water in the soil and perhaps with other factors.

Four 8-inch pots with plants growing in them were placed in the freezing chamber with those described above. The soil in two of the pots was wet and that in the other two dry. Two pots, one with wet and one with dry soil, were removed after 4½ and 5½ hours, respectively. The plants in the wet soil in the pot removed after 4½ hours were uninjured, while those in the dry soil were all dead. The plants

in the dry soil in the pot removed after 5½ hours were also dead, while only 3 of the 10 plants in the wet soil succumbed. The other 7 plants in the wet soil withstood an exposure of 5½ hours to a temperature that killed all the plants in the dry soil in less than 4½ hours. The temperature of the air in the freezing chamber during this test ranged from +1° to -16° C. It reached -9° C. in the first half hour and then went down gradually throughout the experiment. This experiment shows how great an error may be made in interpreting the results of freezing plants under experimental conditions if the soil moisture is not taken strictly into account. No doubt soil texture with its varying water-holding capacity may also influence the results of experimental freezing tests.

AIR AND SOIL TEMPERATURES

Steinmetz (19) states that in Minnesota plants brought from the field in January gave no marked evidence of having been killed during the winter. According to his observations, dead plants first appear at the time of the spring thaw in March. He states that "it may well be questioned whether repeated freezing and thawing occurs under natural conditions in the soil." No doubt he refers to Minnesota conditions, for the soil may freeze and thaw several times during a winter in Kansas. Plants killed by freezing have been seen in Kansas in January and February, and plants badly injured have been found in December. On February 24, 1927, the roots of some nonhardy strains of alfalfa were observed to be entirely softened and disintegrating, having, no doubt, been dead for some time.

Different varieties of alfalfa, including Kansas Common and Grimm, growing in the field showed considerable injury on December 12, 1927, following a drop in the air temperature, as recorded by the thermograph, to -9° F. (-22.8° C.) and the temperature of the soil 2 inches below the surface of 24° F. (-4.4° C.). Frequently serious injury to alfalfa plants is overlooked for a time unless a microscopic study of sections of the root is made. Usually alfalfa in Kansas is not protected by snow during cold periods, as it is in some more Northern States. Although some heaving of the roots occurs, the most serious effects of freezing in this State and in some other States in which observations have been made seem to be the injury produced in the crown and in the upper part of the taproot. The condition of roots badly injured by freezing has been described in detail by the writer (20) ⁵ and by Jones (11). These injuries often so greatly reduce the amount of active tissue that the plant makes little growth and eventually succumbs. These injuries also appear to provide avenues through which microorganisms, especially *Aplanobacter insidiosum* L. McC., may enter and hasten the death of the plant.

As has been stated, the injury to alfalfa plants from freezing is largely limited to the upper part of the taproot and crown. This is due to the fact that the part of the root lower down is protected by the soil. In order to learn more about the rate of temperature fall of the root as compared with that of the air, the following experiment was conducted. A pot in which young alfalfa plants were growing was placed in the freezing chamber. A thermometer was placed in the soil near one of the plants just deep enough to cover the bulb,

⁵ WEIMER, J. L. INJURIES OF ALFALFA ROOTS RESULTING FROM FREEZING. [Unpublished manuscript.]

and a thermocouple needle was inserted into the crown of the plant about 1 cm. above the surface of the soil. A record of the temperature falls in the soil, in the air, and in the plant was kept. These are shown graphically in Figure 4. These data show that the soil temperature lagged behind the air temperature several degrees until the former reached $0^{\circ}\text{C}.$, after which it cooled more rapidly, being only 8 degrees higher than the air temperature at the termination of the experiment. The temperature of the plant took up an intermediate position between that of the air and of the soil. This experiment in-

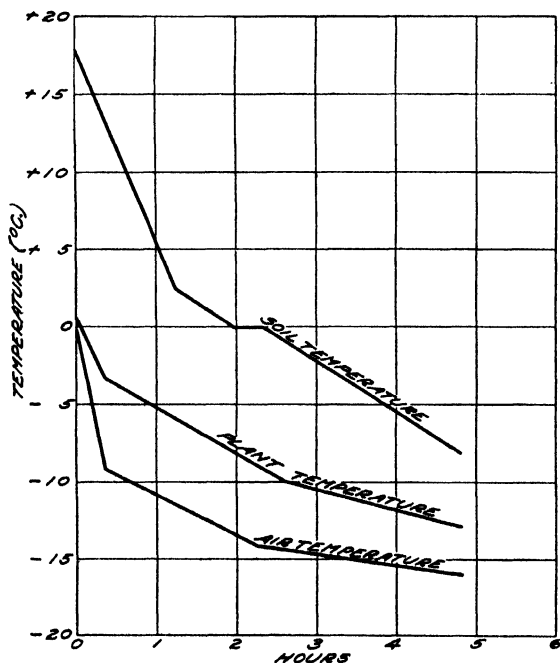


FIGURE 4.—Comparative rate of temperature fall of the soil in an 8-inch pot, of the plant growing in the pot (the record being taken 1 cm. above the soil), and of the surrounding air. The temperatures of the plant and of the soil were originally the same, but by the time the first record was made the plant had cooled down to near the air temperature

icates that the temperature of the alfalfa roots at or slightly above the surface of the soil may drop much more slowly than the air temperature and under some conditions may not reach a temperature as low as that of the air for a long time.

Further information regarding the reason why alfalfa roots freeze only near the surface of the soil is obtained by an examination of thermograph records kept at Manhattan, Kans., since 1926. The soil temperature was taken at a depth of 2 inches. The minimum air and soil temperatures for the coldest periods of the winters 1926-27 and 1927-28 are recorded in Table 5. There were two cold periods

in the winter of 1926-27, one in December and the other in January. The air temperature in December, 1926, did not go much below $0^{\circ}\text{F}.$ ($-17.8^{\circ}\text{C}.$), but the soil temperatures on December 15 and 16 were comparatively low. The air temperature dropped to $-14.5^{\circ}\text{F}.$ ($-25.8^{\circ}\text{C}.$) on January 15, 1927, but the soil temperature went down only to $25.5^{\circ}\text{F}.$ ($-3.6^{\circ}\text{C}.$) owing to a thin covering of snow. There were three comparatively cold periods during the winter of 1927-28, two in December and one beginning on December 31 and extending into January. The first decided drop in temperature came on December 7 and 8, when the air temperatures reached 0° and $-9^{\circ}\text{F}.$ (-17.8° and $-22.8^{\circ}\text{C}.$), respectively. As this cold period was of rather short duration, the soil temperatures did not go very low. However, a histological study of plants from the field showed that

even in the more hardy varieties some splitting along the rays had taken place, while the nonhardy varieties were badly injured. The next cold period, which extended from December 16 to 23, was the most severe, and it seems probable that much of the winterkilling of that season occurred during this time. The minimum air temperature did not go below 0° F. (-17.8° C.) except on two nights during this period, although it approached 0° F. on six of the eight nights. The soil temperature went lower at this time than at any other time during the two years. This may have been due to one of two factors, or to a combination of the two, namely, the duration of the cold period or the dryness of the soil. Only 0.09 inch of rain fell in November and 0.57 inch of rain and 0.07 inch of snow fell in December, 1927, according to the weather records for Manhattan, Kans. It should be noted also that the air temperatures did not go so low as in January, 1927.

TABLE 5.—Minimum air and soil temperatures at Manhattan, Kans., during the coldest periods of the winters of 1926-27 and 1927-28

Date	Minimum air tem- perature	Minimum soil tem- perature 2 inches below surface	Remarks
	° F.	° F.	
Dec. 13, 1926	+4	+25	No snow.
Dec. 14, 1926	0	+20	Do.
Dec. 15, 1926	-2	+17	Do.
Dec. 16, 1926	+7	+18	Do.
Jan. 14, 1927	0	+26	Some snow on ground.
Jan. 15, 1927	-14.5	+25.5	Do.
Dec. 7, 1927	0	+24	No snow; soil very dry.
Dec. 8, 1927	-9	+24	Do.
Dec. 16, 1927	+2	+20	Do.
Dec. 17, 1927	+12	+20	Do.
Dec. 18, 1927	0	+17	Do.
Dec. 19, 1927	-2	+16	Do.
Dec. 20, 1927	-2	+14	Do.
Dec. 21, 1927	+1	+15	Do.
Dec. 22, 1927	+14	+20	Do.
Dec. 23, 1927	+1	+15	Do.
Dec. 31, 1927	-10	+22	Thin layer of snow on ground.
Jan. 1, 1928	-12	+19	Do.
Jan. 2, 1928	-8	+20	Do.
Jan. 3, 1928	-16	+18	Do.
Jan. 4, 1928	-2.5	+19	Do.
Jan. 5, 1928	+8	+20	Do.

The final cold period for the winter of 1927-28 began December 31, 1927, and lasted until January 5, 1928. The air temperatures during this period were the lowest experienced during the two years, but the plants were protected somewhat by a thin layer of snow, which kept the soil temperature from going as low as it had done previously.

The data in Table 5 show that the temperature of the soil at a depth of 2 inches remained very much higher than that of the air during the coldest periods experienced at Manhattan in the two winters during which records have been kept. An examination of alfalfa plants of different varieties showed that on January 14 India was entirely killed and several other varieties such as Arizona, South African, Spanish, Smooth Peruvian, and Hairy Peruvian were severely injured. More or less splitting of the rays or other parts of the roots was seen in most of the varieties studied. When growth began in the spring, however, only those varieties listed above showed much winterkilling. It should be added that there was only a moderate

amount of winterkilling at Manhattan during the winter of 1926-27 and also during the following winter.

BOUND WATER AS A MEASURE OF COLD RESISTANCE

Rosa (18) and Lott (13) have used the dilatometer method for determining the amount of nonfreezable water in plant tissue. Both report having found a correlation between the amount of non-freezable or bound water and the hardness of the tissues with which they worked. Steinmetz (19), using a method described by Newton and Gortner (17), found no correlation between the bound water and winter hardness in alfalfa plants.

Following the method described by Rosa and Lott, the writer tested a number of alfalfa roots of different varieties in the fall and again in the following February. The plants were all from a plot in which the different varieties were growing in rows. The soil was fairly uniform in texture and in drainage. When an experiment was begun the plants were brought to the laboratory, washed, dried, cut into thin slices, and 10-gm. samples weighed into tared weighing bottles. The tissue was covered with petroleum ether in tightly stoppered bottles and held in the ice box until needed, which was usually not longer than 24 hours. Preliminary tests indicated that the amount of bound water in the samples did not change when held under these conditions for several hours. In most cases all the samples of a series were collected and prepared for use at the same time. However, in one experiment the samples were collected and tested on the same day. A composite sample of 10 to 15 plants was used in each case. The dilatometer was immersed in a salt and ice bath held at about -5°C . Difficulty was experienced in inoculating all the samples at the same temperature. Usually the nonhardy varieties became inoculated at about -2°C . The writer discovered no way which would consistently start the formation of ice crystals in some of the more hardy samples until they had cooled one-half degree or more. This did not always happen, but when it did sometimes a larger amount of water froze than in the nonhardy varieties. At other times, however, only a small amount of water froze, even in samples which did not become inoculated until a temperature of -3°C . was reached.

An examination of Table 6 shows that the hardened plants had a slightly higher water content than the nonhardened ones with the exception of Grimm, Kansas Common, and Spanish. In every case, however, more of the water in the nonhardened plants froze, leaving a smaller amount of unfrozen or bound water. There appears to be, therefore, a correlation between the bound water and the state of hardening of the plants when the two separate groups of plants are considered. However, when the individual varieties within the groups are compared no such correlation can be seen. Of course, no correlation was expected in the nonhardened group. It scarcely seems probable that the plants frozen in February were not hardened, as they were taken from soil frozen 2 inches or more in depth. It must be admitted, however, that little is known as yet regarding the actual time required for the hardening of alfalfa roots. The varieties shown in Table 6 are so arranged that the more hardy appear toward the top and the least hardy lower down. The least

hardy group comprises the five varieties listed last in the table. Hardigan, Cossack, and Grimm are usually considered among the most hardy. The remaining varieties may be considered as intermediate, although some of them are also rather hardy. Of those frozen in February, Grimm, of the hardy group, contained the highest percentage of bound water, while Spanish, Smooth Peruvian, and nonhardy varieties ranked next. On the other hand, Cossack, a hardy variety, had the least bound water. Hardiness in these experiments is not necessarily correlated with low moisture content, for, as pointed out, the plants of the hardened series contained slightly more water than the others. On the other hand, Lott (13) found that raspberry plants having a lower moisture content also had a higher percentage of bound water. Rosa (18) likewise obtained an increase in bound water with a decrease in the water content of the tissue.

TABLE 6.—Quantity and state of the water in 10-gram samples of different varieties of alfalfa, when hardened and nonhardened

(Hardened plants frozen February, 1928; nonhardened ones September, 1927)

Variety	Hardened					Nonhardened				
	Dry weight	Total water	Freezable water	Bound water	Percentage of bound water	Dry weight	Total water	Freezable water	Bound water	Percentage of bound water
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>		<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	
Hardigan.....	3.070	6.930	1.5	5.430	78.354	3.488	6.512	2.9	3.612	55.466
Cossack.....	2.970	7.030	2.8	4.230	60.170	3.121	6.879	3.6	3.279	47.666
Grimm.....	2.837	7.163	1.0	6.163	86.039	2.809	7.191	3.4	3.791	52.718
Lebeau.....	2.788	7.212	1.4	5.812	80.587	2.807	7.193	3.75	3.443	47.865
Turkestan.....	1.726	8.274	1.5	6.774	81.870					
Kansas Common.....	2.755	7.245	1.8	5.445	75.155	2.375	7.625	4.0	3.625	47.540
Dakota.....	2.708	7.232	1.7	5.532	76.493	2.843	7.157	3.8	3.357	46.905
Utah.....	2.944	7.056	1.4	5.656	80.158	3.022	6.978	3.2	3.778	54.141
Argentine.....	2.758	7.242	1.6	5.642	77.906	2.795	7.205	4.1	3.105	43.005
Italian.....	2.799	7.201	2.3	4.901	68.059	2.959	7.041	3.5	3.541	50.291
Spanish.....	2.622	7.378	1.3	6.078	82.380	2.327	7.673	4.15	3.523	45.914
South African.....	2.930	7.070	1.7	5.370	75.954	3.503	6.497	3.3	3.197	49.207
Smooth Peruvian.....	3.003	6.997	1.1	5.897	84.278	3.185	6.815	3.15	3.665	53.778
Hairy Peruvian.....	2.936	7.064	2.05	5.014	70.979	2.992	7.008	3.1	3.908	55.764

CONCLUSIONS

It may be concluded from the results of these investigations that there is no correlation between the freezing point of alfalfa root tissue when in a hardened condition and its known winter hardiness. The freezing point of roots in a nonhardened condition is often lower than that of hardened roots. Likewise, the freezing point of the root tissue of nonhardy alfalfa varieties is frequently lower than that of hardy varieties under apparently the same conditions.

It is possible to distinguish between plants in a hardened and in a nonhardened condition by freezing the roots of plants growing in pots or by removing the roots from the soil and freezing them.

The amount of bound water in the tissue of hardened and nonhardened alfalfa roots may serve as a measure of their degree of hardening, but it does not necessarily differentiate between hardy and nonhardy varieties.

SUMMARY

The freezing point of alfalfa root tissue was determined by the thermoelectric method. In these tests there was little correlation between the freezing points and the diameters of the roots.

There was a slight correlation between the freezing points of sections of alfalfa taproots taken near the crown and similar sections taken 10 cm. below.

The average freezing point of root tissue in or near the cambium was -2.11°C ., while that of the center of the root was -2.3°C .

No correlation was found to exist between the freezing point and the known hardness of the root tissue of different varieties of alfalfa.

Alfalfa roots under the conditions of these experiments when growing in 8-inch pots were killed in $3\frac{1}{2}$ to 5 hours at temperatures ranging from -20° to -25°C . and withstood average temperatures of -6.5° to -9.2°C . for more than 7 hours. Hardened plants from the field and nonhardened plants from the greenhouse withstood temperatures of -21° to -26°C . for 6 and $3\frac{1}{2}$ hours, respectively.

Many roots were removed from the soil before they were exposed to freezing temperatures. When this was done, small nonhardened roots were killed in 15 minutes at temperatures from -17° to -20°C ., while larger roots withstood these temperatures twice as long. The time of exposure necessary to kill roots of small plants varied from 15 minutes to $3\frac{1}{2}$ hours, depending upon the temperature to which they were exposed. The period during which field plants endured temperatures of -17°C . and -23.5°C . (range -21° to -26°C .) varied from 50 minutes to $3\frac{1}{2}$ hours, depending upon their degree of hardening.

The amount of cold which alfalfa roots can withstand depends upon their hardness, the amount of insulation afforded by the substratum in which they are embedded, soil moisture, and other factors.

Roots of nonhardened plants with abundant carbohydrate reserves freeze less easily than roots of plants that have been deprived of light for some time. Presumably this difference is due to a change in the physiological condition of the cells brought about by a lack of root reserves.

It is shown by using the dilatometer method that a correlation existed between the amount of bound water and cold resistance of hardened and nonhardened plants. A similar correlation with respect to the known hardness of different varieties, however, was not evident.

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RELATION OF TEMPERATURE OF FERMENTATION TO QUALITY OF SAUERKRAUT¹

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INTRODUCTION

The importance of temperature control in sauerkraut manufacture is becoming better appreciated in factory practice, but until now only cursory observations, rather than accurate measurements under well-regulated conditions, have been made. Since the temperature of cabbage as it comes into the factory may vary from as high as 85° to as low as 32° F., depending upon weather conditions, temperature control has been adopted in many factories. Great differences of opinion exist among sauerkraut packers, however, as to the best temperature at which to carry out the fermentation. Some prefer to operate at a temperature of 70° to 80°, while others let the temperature fall as low as 50°. The higher temperature has the advantage of inducing a rapid formation of acid, but this is offset by a tendency to produce soft and pink sauerkraut. For this reason a temperature must be selected which gives a rapid fermentation without sacrificing the quality of the product.

REVIEW OF LITERATURE

The literature on the relation of temperature to sauerkraut fermentation is both meager and conflicting. Wehmer³ recommends a temperature of 40° F. Henneberg⁴ suggests that 68° to 77° be employed at the start and 50° to 59° during the later stages of the fermentation. Fabian⁵ recommends that the fermentation be carried out at 80° in order to give a product of pleasant taste and aroma. LeFevre⁶ reports that sauerkraut can be made in from six to eight days by employing a temperature of 80°. The present authors have never been able to make sauerkraut of good quality at this temperature. It was possible to obtain a sufficiently high acid concentration in a short time at this temperature, but the product still possessed the characteristic odor and flavor of raw, sour cabbage rather than that of sauerkraut. At elevated temperatures (75° to 80°) there is also serious danger of producing soft and slimy sauerkraut and at times pink sauerkraut. In a previous study of the relation

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³ WEHMER, C. DIE SAUERKRAUTGÄRUNG. *Centbl. Bakt. [etc.]* (II)10: [625]-629. 1903.

⁴ HENNEBERG, W. DAS SAUERKRAUT (SAUERKOHLE). 89 p. illus. *Inst. Gärungsgewerbe*, Berlin. 1916. [Reprint from *Deut. Essigindustrie Jahrg.* 20: [133]-136, [141]-144, 152-155, 160-161, 166-170, 176-177, 184-185, 192-194, 199-202, 207-209, 215-216, 223-225, illus. 1916.]

⁵ FABIAN, F. W. THE SCIENCE OF MAKING SAUERKRAUT. CARE NEEDED TO EXCLUDE UNDESIRABLE BACTERIA FROM KRAUT—HOME-MADE STARTER MAY BE USED. *Mich. Agr. Expt. Sta. Quart. Bul.* 9: 50-51. 1926. [Also *Market Growers' Jour.* 41: 639. 1927.]

⁶ LEFEVRE, E. THE COMMERCIAL PRODUCTION OF SAUERKRAUT. *U. S. Dept. Agr. Circ.* 35, 30 p., illus. 1928.

of temperatures to commercial sauerkraut production, it was reported ⁷ that the best sauerkraut was produced when temperatures between 55° and 65° existed in the vat. The experiments recorded in this paper are a continuation and extension of the previous work, and the results now presented substantially confirm it as to the best temperatures at which to produce sauerkraut.

EXPERIMENTAL METHODS

LABORATORY EXPERIMENTS

The sauerkraut was made either in 10-gallon stone jars or in 45-gallon barrels. Early Copenhagen and All Seasons cabbage were used. Sixty pounds of cabbage were packed in the jars and 300 pounds in the barrels. Two and one-half per cent of salt was used in all cases. The following three ranges of temperature were studied: -10° to 50°, 55° to 60°, and 70° F.

The fermentations at 70° F. were carried out in a greenhouse where the temperature was maintained by means of thermostat control. The fermentations at 55° to 60° were conducted in a basement, the temperature being regulated by means of steam heat and air circulation. The low-temperature fermentations, -10° to 50°, were made in barrels placed outside of the building where they remained from December to March.

TABLE 1.—*The effect of fermentation temperature on the quality of sauerkraut*

Sample No.	Size of container	Temperature of fermentation	Age of sauerkraut	Final acidity as lactic acid	Quality
		° F.	Days	Per cent	
1	10-gallon jar	60	43	1.98	Good.
2	do.	60	43	1.92	Do.
3	do.	70	43	2.02	Fair.
4	do.	70	43	1.91	Do.
5	45-gallon barrel	-10 to 50	125	.58	Very poor.
6	do.	-10 to 50	125	.58	Do.
7	do.	55 to 60	35	1.67	Good.
8	do.	55 to 60	35	1.72	Do.
9	do.	70	35	1.62	Poor.
10	do.	70	35	1.62	Do.

Table 1 summarizes the results of the experiments. The sauerkraut made at 70° F. was only fair in quality; that which was made at 55° to 60° was good in every instance, and that which stood outside did not become sauerkraut at all but was merely spoiled cabbage. This material was rotted to the depth of a foot or more at the top and at the bottom, was raw and contained no appreciable amount of acid. Although such extreme conditions do not prevail in the making of sauerkraut the results show in an exaggerated form some of the effects which may be expected when sauerkraut is made at low temperatures.

FACTORY EXPERIMENTS

Other experiments were conducted in a commercial sauerkraut factory. The cabbage used was the All Seasons variety and had been stored outside for about a month in a well-ventilated hay-covered pile

⁷ PARMELE, H. B., FRED E. B., PETERSON, W. H., MCCONKIE, J. E., and VAUGHN, W. E. RELATION OF TEMPERATURE TO RATE AND TYPE OF FERMENTATION AND TO QUALITY OF COMMERCIAL SAUERKRAUT. Jour. Agr. Research 35: 1021-1038, illus. 1927.

and had a temperature of about 38° F. when brought to the factory for cutting. As it left the cutter it fell through a chute where it was heated by means of a steam jet to the particular temperature desired. The plan followed was to fill three pairs of vats with cabbage at approximately 42°, 60°, and 70°, respectively. Each pair of vats was filled simultaneously so as to have the same grade of cabbage in both. Of the six vats used in the experiment Nos. 2, 6, 5, and 7 were filled the same day with 9 tons of cabbage and Nos. 3 and 9 were filled a day later with 6 tons.

Three copper-constantan thermocouples were put into each vat; one 2 feet from the bottom and the same distance from the side of the vat, another in the center, and the third 2 feet from the top and a like distance from the outside of the vat, but opposite the bottom thermocouple. The cold junction of the thermocouples was kept in ice water in a Dewar flask and the temperature of the junction in the vat read on a Leeds-Northrup potentiometer graduated directly in degrees Fahrenheit.

Another method of obtaining temperatures was used with four of the vats. A bamboo pole, the intersections of which had been bored out, was placed in the middle of the vat at the time of filling. In this tube were suspended thermometers at heights of 2 feet and 4 feet from the bottom. To prevent a change in the reading while the thermometer was being withdrawn and read the lower end was inclosed in a small tube filled with water.

In order to obtain samples of sauerkraut juice small Büchner funnels were attached to thick-walled rubber tubes and were placed in the vats near the top and bottom thermocouples. The sample was drawn into a receiving bottle by means of a hand-operated suction pump. The first portion of the juice was discarded to insure that the sample used for analysis was obtained from the vat and was not juice that had been standing in the tube. Titratable acidity, number of bacteria, and time of methylene-blue reduction were determined on each sample. The methods used for these determinations, and also for the analysis of the sauerkraut, have been described in a previous publication.⁸

TABLE 2.—Temperature of sauerkraut in factory vats as determined by thermometers at various times during the fermentation

Age of sauerkraut (days)	Temperature of factory (° F.)	Temperature (° F.) in—							
		Vat 2		Vat 6		Vat 3		Vat 9	
		Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
3	45	43	41	43	41	—	—	57	59
4	48	42	45	42	43	60	54	62	62
6	50	44	46	42	45	62	58	62	63
7	60	44	48	43	45	64	59	64	63
9	50	45	48	44	46	63	58	64	64
10	38	45	48	46	46	62	57	64	65
14	54	48	50	—	50	59	54	—	—
17	55	50	53	52	54	60	57	—	—
23	57	52	53	53	54	61	59	—	—
31	52	53	50	52	52	60	57	—	—
35	55	52	51	52	51	56	53	—	—

⁸ PARMELE, H. B., FRED, E. B., PETERSON, W. H., McCONKIE, J. E., and VAUGHN, W. E. Op. cit.

The results of the observations and the analyses are recorded in the accompanying illustrations and tables. The temperatures obtained by means of thermocouples are given in Figures 1 to 3 and those taken with thermometers are recorded in Table 2. The results in most cases do not differ by more than 1° F. Part of this difference is due to the fact that the paired thermometer and thermocouple were a foot or more apart. Unfortunately, because of a change in operation, the last five readings of the thermocouples were erroneous and had to be discarded.

FERMENTATION AT HIGH TEMPERATURES (67° TO 75° F.)

The first sign of fermentation was the evolution of gas. A thick layer of foam formed on the surface of the vat during the first night.

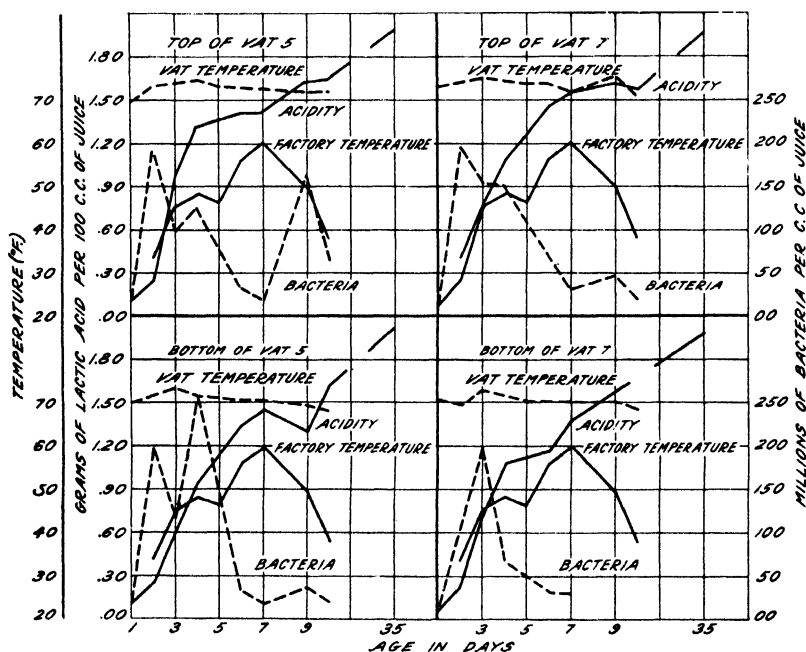


FIGURE 1.—Temperature, acidity, and number of bacteria in vats 5 and 7 at various times during fermentation of sauerkraut

This did not occur on the vats fermented at a medium temperature until after three days and did not occur at all on the vats fermented at low temperatures.

Figure 1 shows that in general the temperature in vats 5 and 7 rose steadily from the first day to the third or fourth day. This rise in temperature can not be attributed to conduction of heat into the vat from the outside, for the vats were approximately 26° F. above factory temperature at the time they were filled. The production of heat must be caused either by plant-cell respiration or by bacterial activity. The latter was perhaps the most important cause, since the rise in temperature coincided with the time at which the bacteria were present in greatest numbers.

The number of bacteria reached a maximum about the second or third day, which was the time when the acidity began to increase rapidly. This increase was greatest during the third and fourth days. As the acid accumulated the number of bacteria decreased. The type of bacteria also changed after the third day. During the first two or three days coccus forms and short rods predominated, but after three days these gave way to large rods, which persisted throughout the remainder of the fermentation.

FERMENTATION AT MEDIUM TEMPERATURES (57° TO 64° F.)

On the first day the temperature in vats 3 and 9 (fig. 2) fell below the initial temperature of the vat. This was probably due to inequalities

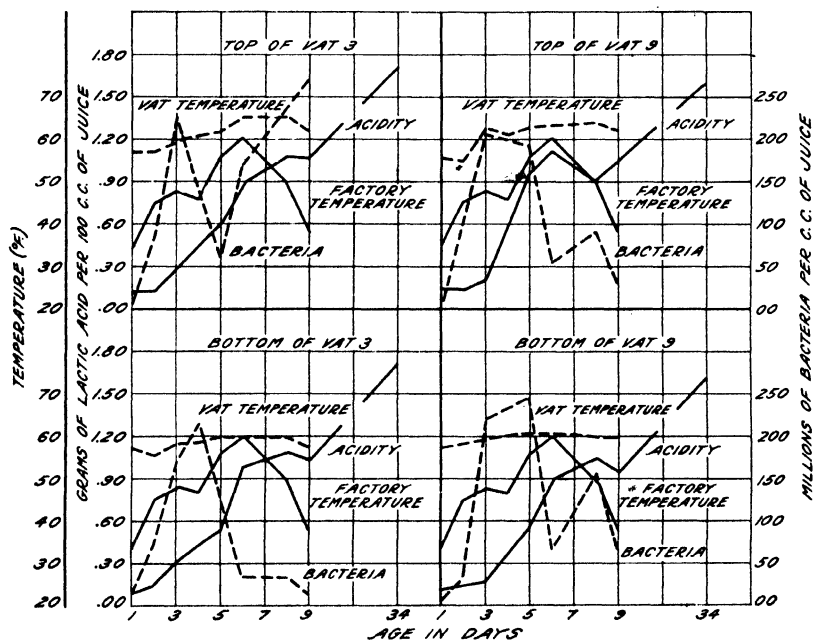


FIGURE 2.—Temperature, acidity, and number of bacteria in vats 3 and 9 at various times during fermentation of sauerkraut

in the temperature of the cabbage in the vicinity of the thermocouple. The temperature began to rise on the second day and continued to do so for five days after which it remained constant until the eighth day. This rise in temperature may be taken as further evidence of the bacterial origin of the heat developed, for it occurred coincidentally with the rise in acidity and the increase in the number of bacteria. If the formation of heat was due to plant-cell respiration the temperature would have risen from the start and would not have been delayed until the second day.

FERMENTATION AT LOW TEMPERATURES (40° TO 46° F.)

The outstanding facts shown by Figure 3 are that at the low temperature of vats 2 and 6, no appreciable action took place. The gradual rise in temperature was probably the result of heat taken up from the

atmosphere. Acid production was low and the numbers of bacteria were small throughout the fermentation. These results are in agreement with those of experiments previously reported on the production of sauerkraut at low temperatures.⁹

CHANGES IN TIME OF METHYLENE-BLUE REDUCTION

The time of methylene-blue reduction may be used as a rough measure of bacterial activity, activity being inversely proportional to the time of reduction, at least approximately. In Table 3 are recorded the changes in the time of methylene-blue reduction of the juice from each of the vats. A comparison of these data with those in

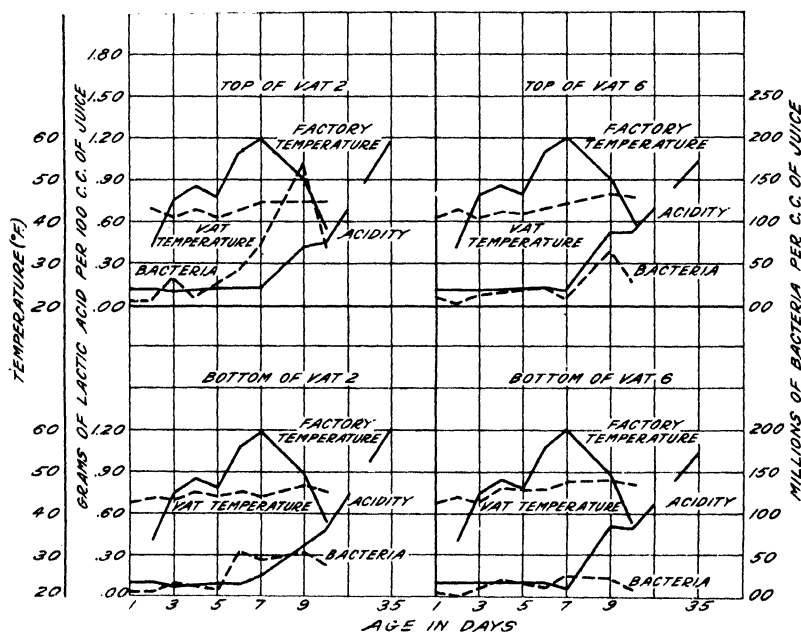


FIGURE 3.—Temperature, acidity, and number of bacteria in vats 2 and 6 at various times during fermentation of sauerkraut

the illustrations shows that the time of reduction fell from a maximum of over 2 hours at the beginning of the fermentation to a minimum of 15 minutes at the end of 3 to 10 days. The minimum time was reached first by those vats which had the highest initial temperature, i. e., the vats in which fermentation was most active.

COMPOSITION AND QUALITY OF THE SAUERKRAUT

The vats at high and medium temperatures were opened at the end of 35 days. The low-temperature vats contained so little acid at this time that they were left to ferment and were first opened at the end of 158 days. Samples were taken from the top, the center, and the bottom of each vat. Table 4 gives the results of the analysis and grading.

⁹ PRIEM, L. A., PETERSON, W. H., and FRED, E. B. STUDIES OF COMMERCIAL SAUERKRAUT WITH SPECIAL REFERENCE TO CHANGES IN THE BACTERIAL FLORA DURING FERMENTATION AT LOW TEMPERATURES. *Jour. Agr. Research* 34: 79-95, illus. 1927.

TABLE 3.—Changes in time of reduction of methylene blue by sauerkraut juice from vats kept at different temperatures during fermentation

Age of sauerkraut (days)	Time of reduction in minutes for juice from—											
	Vat 2, 42° F. ^a		Vat 6, 42° F. ^a		Vat 3, 57° F. ^a		Vat 9, 57° F. ^a		Vat 5, 70° F. ^a		Vat 7, 70° F. ^a	
	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top
1	(^b)	(^b)	(^b)	(^b)	(^b)	(^b)	(^b)	(^b)	(^b)	(^b)	(^b)	(^b)
2	(^b)	(^b)	(^b)	(^b)	105	(^b)	(^b)	(^b)	80	80	90	90
3	(^b)	(^b)	(^b)	(^b)	15	15	15	15	30	30	15	15
4	(^b)	(^b)	(^b)	(^b)					15	15	15	15
5					15	15	15	15				
6	105	(^b)	(^b)	(^b)	15	15	15	15	105	15	15	15
7	(^b)	(^b)	(^b)	(^b)					15	15	15	15
8					15	15	15	15				
9	60	60	120	90	90	45	45	45	30	30		15
10	15	15	15	90					45	45		60

^a Initial temperature of fermentation.^b Not reduced in two hours.

TABLE 4.—Age, quality, and composition of sauerkraut from experimental vats kept at various fermentation temperatures

Vat and initial temperature	Age	Moisture	Acetic acid	Lactic acid	Ethyl alcohol	Reducing sugars as glucose	Quality of sauerkraut
	Days	Per cent	Per cent	Per cent	Per cent	Per cent	
Vat 2, 42° F.	158	90.5	0.249	1.406	0.282	0.15	Poor.
Vat 6, 42° F.	158	89.5	.420	1.576	.326	.12	Do.
Vat 3, 57° F.	34	90.2	.371	1.442	.281	.12	Excellent.
Vat 9, 57° F.	34	91.9	.332	1.462	.259	.11	Good.
Vat 5, 70° F.	35	92.1	.408	1.503	.331	.12	Fair.
Vat 7, 70° F.	35	90.6	.420	1.513	.331	.15	Do.

The quality of the sauerkraut which was produced at the highest temperature was only fair judged from the standpoint of flavor, color, and texture. High temperature undoubtedly favors the production of sauerkraut of soft texture and pink color, for numerous soft and pink portions of sauerkraut were found in both vats. It has also been demonstrated in the laboratory that fermentations conducted at high temperatures tend to produce pink sauerkraut. The finest grade of sauerkraut was obtained from the vats kept at intermediate temperatures. The sauerkraut from these vats was white and crisp throughout. The cabbage fermented at the lowest temperature produced sauerkraut that was poor in quality, and possessed a bitter taste, poor texture, and a dark color.

The sauerkraut from the different vats showed no essential difference in percentage content of fermentation products. The quality of sauerkraut depends upon something other than mere acidity. While a proper degree of acidity may be taken as the first requisite, there are other products, as yet not measured, which distinguish a sauerkraut of good flavor from one of poor flavor.

SUMMARY

The quality of sauerkraut was found to depend very largely upon the temperature at which fermentation was carried out, the most favorable temperature for fermentation was between 60° and 65° F.

High temperatures favored the production of soft and pink sauerkraut.

A rise of 3° to 5° F. occurred during the first eight days in fermentation vats kept at temperatures above that of the surrounding air. This rise in temperature was coincident with the greatest activity of the bacteria and is believed to have been caused by bacterial action.

COMPARATIVE VALUE OF THE SIZE OF PHYTOPHTHORA SPORANGIA OBTAINED UNDER STANDARD CONDITIONS¹

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INTRODUCTION

Rosenbaum (25)³ was perhaps the first to make a comprehensive effort to evolve unity from the chaotic morphological status of the genus *Phytophthora*. That his efforts failed materially to influence other workers can be attested by the fact that a large number of so-called new species have been described since the appearance of his paper. This is not at all surprising because a great many investigators still cling to the idea that the species is rigid and immutable. It is perhaps for this same reason that in his work Rosenbaum used only one strain from each species; had he used a dozen different strains, his conclusions would have been remarkably different and his taxonomic key decidedly less simple.

Although the senior writer (14) has demonstrated remarkable deviations among the different strains of the same species of *Phytophthora* affecting shape and size of sporangia, presence and absence of sexual bodies, macroscopic characters of colonies, microscopic nature of submerged hyphae, and even pathogenicity, slight differences have still been used by different investigators as comprehensive bases for the creation of new species.

Since environmental factors exert a great influence upon the morphology of *Phytophthoras*, and since the size and shape of sporangia constitute the principal basis for the morphological classification of this genus, it becomes obvious that uniform environmental conditions are imperative for dependable measurements. It is not only a useless effort to describe a *Phytophthora* directly from its host, but it may prove a misleading procedure as well, because a given organism may be described as a different species according to the particular host which it happens to have infected. The complex vegetable media are but little more desirable substrata than the host plant, because not all varieties of oats, carrots, potatoes, plums, and all the heterogeneous constituents of the phytopathologists' agars have the same chemical or physical composition. There can be no dependable morphological classification of *Phytophthoras* without standardized environmental conditions. But as yet no such uniformity exists, as can be seen from the literature on *Phytophthoras*. The men who described these organisms worked in different countries, under different climatic and laboratory conditions, and with media that can not be standardized or duplicated. As a result, the literature is teeming with conflicting statements.

¹ Received for publication Mar. 8, 1929, issued August, 1929. Scientific Paper No. 77 of the West Virginia Agricultural Experimental Station.

² The writers express their thanks to Russell Brown for his help in biometrical computations

³ Reference is made by number (italic) to "Literature cited," p. 309.

SIZES OF SPORANGIA RECORDED IN THE LITERATURE

In order to show the confusion which now exists in the literature, the writers have listed in Table 1 the sizes of sporangia as given by some representative workers with this genus. Even a cursory glance is sufficient to show the amazing lack of consistency throughout the list. But just how much of this is caused by an absence of uniformity in technic and environmental conditions, and how much by the normal differences in the different strains and mutants of the same species, may never be known.

The average size, whenever given, is selected and given in Table 1 as the more comprehensive measure.

TABLE 1.—Review of sporangial measurements of *Phytophthoras* as noted by other workers

Organism	Investigator	Measurements in microns
<i>P. allii</i>	Tanaka (35)	36.5 by 49.4.
	(Coleman (5))	20.6 by 30. 45.4 by 51.2. 43.3 by 71.
<i>P. arecae</i>	Wilson (38)	20.6-45.4 by 30.1 by 71.
	Rosenbaum (25)	47.92 by 30.05.
	Sundaraman and Ramakrishnan (32)	27-42 by 32-64.
	(Cohn and Lebert (25))	48 by 35-68.
	Schroeter (25)	35-40 by 50-60.
	Saccardo (28, p. 237-238)	35-40 by 50-90.
	Hartig (24 and 25)	25-40 by 25-40.
	Osterwalder (25)	14.04-24.4 by 119.56.
	Zimmerman (25)	17-30 by 25-60.
	Hori (25)	30-50 by 50-60.
	Bubak (25)	15-25 by 15-120.
	Van Hook (25)	30-42 by 40-58.
<i>P. cactorum</i>	Lafferty and Pethybridge (10)	127 by 37 (oatmeal agar).
	Stevens and Plunkett (31)	30 by 52 (raw apple tissue).
	Rosenbaum (24)	21-29 by 22-32.
	do	27 by 34.5.
	Beach (1)	26.06 by 36.62.
	do. (1)	24.9 by 34.4 (apple strain).
	do. (1)	25.97 by 32.34 (first of rhubarb strain).
	do. (1)	27.5 by 37 (second of rhubarb strain).
	do. (1)	25.2 by 33.8 (third of rhubarb strain).
	Rose (22)	25.09 by 32.26.
	Rose and Lindgren (23)	26.8 by 34.4 (artificial culture).
<i>Belpharospora cambivera</i>	Petri (19)	36.9 by 40.3 (diseased fruits).
<i>P. capsici</i>	Leonian (11)	40-54 by 60-75.
<i>P. cinnamomi</i>	Rands (20)	36 by 60.
	Smith and Smith (29)	33 by 57.
<i>P. citrophthora</i>	Carne (3)	35 by 50.
	Wilson (38)	19-37 by 19-60 (average 30 by 38.5).
<i>P. colocasiae</i>	Butler and Kulkarni (7)	18-26 by 30-60, or larger.
	McMurphy (15)	18-26 by 38-60.
	MacMillan (18)	30-42 by 42-78.
<i>P. erythroseptica</i>	Wilson (38)	20 by 32.
	Rosenbaum (25)	20 by 30.
	Wilson (38)	27.65 by 44.85.
	Tucker (36)	25 by 80, rarely 42 by 80.
	Reinking (31)	30.95 by 52.67.
	McRae (16)	31.28 by 52.27.
<i>P. faberi</i>	do.	16-36 by 34-70 (cacao fruit).
	Von Faber (5)	25-32 by 32-66 (Hevea fruits).
	Rosenbaum (25)	25-30 by 42-80.
	Rosenbaum (17)	32.29 by 48.52.
	Ocfemia and Roldan (17)	25.5-43.49 by 37.5-59.49.
	Wilson (38)	29.45 by 44.71.
<i>P. fagi</i>	Lafferty and Pethybridge (10)	15-30 by 30-40.
	Rosenbaum (25)	31 by 56 (raw apples).
		31 by 45 (artificial media).
<i>P. hibernalis</i>		25.67 by 33.65.
	Carne (3)	16.1 by 34.6 (lemon leaves, on potato-dextrose agar).
		16.4 by 34.6 (leaf and fruit tissues).
		14.3 by 30.3 (potato-dextrose agar).
<i>P. infestans</i>	(Saccardo (26))	15-20 by 27-30.
	Berlese (2)	16-24 by 22-32.
	Clinton (4)	11-20 by 17-35.
	Rosenbaum (25)	18.27 by 27.08.

TABLE 1.—Review of sporangial measurements of *Phytophthoras* as noted by other workers—Continued

Organism	Investigator	Measurements in microns
<i>P. jatrophae</i>	Rosenbaum (25).....	37.55 by 49.65. 21 by 48 (Hevea fruits).
<i>P. meadii</i>	McRae (18).....	30 by 48 (French-bean agar). 23 by 32 (Aqua submersis).
<i>P. melongenae</i>	Tanaka (53).....	33.9 by 42.4.
<i>P. mexicana</i>	Hotson and Hartge (9).....	16-33 by 16-77 (artificial culture). 24.2 by 46.2 (tomato fruit).
<i>P. nicotianae</i>	Van Breda de Haan (35).....	25 by 36.
	Rosenbaum (25).....	29.95 by 37.58.
	Tisdale and Kelley (35).....	25.3 by 36.67 (Holland strain). 28.98 by 38.2 (Java strain). 15.75 by 21-38 by 56 (oatmeal agar). 32 by 51.6 (tobacco plant).
<i>P. omnivora</i>	Berlese (2).....	35 by 50-60.
	Stevens (30).....	35-40 by 50-60.
	Wilson (38).....	17-30 by 20-60.
	Dastur (35).....	20-40 by 25-50.
	Wilson (38).....	10-45 by 16-60.
<i>P. parasitica</i>	Tisdale and Kelley (35).....	25.86 by 31.25.
	Rosenbaum (25).....	23.39 by 43.64.
	Dastur (6).....	10-15 by 13-20 (Vinea rosea).
	Godfrey (7).....	21-42 by 27-54.
<i>P. parasitica rhei</i>	Leonian (13).....	33.2 by 43.7 (Type I).
		36.7 by 43.7 (Type II).
		37.3 by 43.7 (Type III).
		21 by 25.5 (Type IV).
		28 by 35 (Type V).
<i>P. phaseoli</i>	Saccardo (27, p. 341).....	20-24 by 35-60.
	Clinton (4).....	17-27 by 28-43.
	Rosenbaum (25).....	19.05 by 27.87.
<i>P. pini</i>	Leonian (12).....	35 by 55.5.
	Gravatt (8).....	19-40 by 28-86.
	Wilson (38).....	30-32 by 40-74.
<i>P. syringae</i>	Rosenbaum (25).....	25.33 by 39.86.
	Lafferty and Pethybridge (10).....	26 by 38 (Klebahn's strain). 27 by 40 (apple strain).
<i>P. terrestris</i>	Sherbakoff (28).....	30.5 by 42.5.
<i>P. thalictri</i>	Wilson (38).....	13-17 by 20-27.
<i>P. theobromae</i>	Thurston and Orton (34).....	16.7-22.3 by 20.4-29.7.
	Von Faber (5).....	25-42 by 30-80.

Twenty-one different measurements are recorded in Table 1 for *Phytophthora cactorum*. Obviously a measurement which reads 15-25 by 15-120 microns is meaningless because almost any organism may be included in such a broad sphere. Saccardo's 35-40 by 50-90 microns, or Schroeter's 35-40 by 50-60 microns are decidedly different from Rosenbaum's measurement of 27 by 34.5 microns, or Beach's 24.9 by 34.5 microns. *P. erythroseptica* recorded by Wilson as measuring 20 by 30 microns materially differs from Rosenbaum's 27.65 by 44.85 microns.

If size is to be used as a dependable factor in taxonomy, such glaring contrasts in the measurements of different investigators must first be eliminated. Standardized conditions whereby all workers use the same simple media, and the same environmental conditions regardless of their localities, should help considerably in the elimination of doubtful factors. With this purpose in mind, the writers undertook to work out the size factor in the organisms listed below.

Information regarding the following group of 48 organisms is given by the first author (12 pp. 444-447):

- Phytophthora arecae* (Colem.) Pethyb.
- P. cactorum* 1 (Lebert and Cohn) Schrot.
- P. cactorum* 2 (Beach).
- P. cactorum* 3.
- P. capsici* Leonian.

P. citrophthora Smith and Smith.

P. crytogea Pethyb. and Laff.

P. fagi (Hartig) Hartig.

The infestans group:

P. infestans (Mont.) De Bary.

P. phaseoli Thaxter.

P. mexicana Hotson and Hartge.

P. nicotianae Breda de Haan.

The omnivora group:

P. colocasiae Racib.

P. faberi Maubl.

P. palmivora (Butl.) Butl.

P. parasitica Dastur.

P. parasitica rhei I Godfrey.

P. parasitica rhei II.

P. parasitica rhei III.

P. parasitica rhei IV.

P. parasitica rhei V.

P. sp. Reddick's I.

P. terrestris Sherb.

P. pini Leonian--

A collection of tropical Phytophthoras sent by Carl Hartley as follows:

8, 22, 26, 36, 44, 97, 100, 102, 116, 117, 121, 123, 126, 130, 136, 137, 138, 139, 140, 141, 142, 143, 144, and 145.

P. sp. from Byrophyllum; collected and isolated by A. Berg in Bermuda.

P. sp. from tobacco; sent to Leonian by Tisdale.

P. sp. from tomato; isolated by Leonian at Morgantown, W. Va.

P. sp. from Vinca; isolated by Leonian; last material furnished by E. F. Smith.

The foregoing four organisms have been identified as *P. omnivora*.

The following group of organisms was sent by H. S. Fawcett:

No. 760, from tomato; isolated by Sherbakoff.

No. 846, from citrus, southern California.

No. 1026, from orange fruit, Riverside, Calif.

No. 1216, sent from Amsterdam as *P. parasitica*.

No. 1245, from citrus, southern California.

No. 1246.

No. 1274, from citrus, southern California.

No. 1287, from citrus, southern California.

No. 1290 b, from citrus tree, Corsica.

No. 1290 d, "Supposed to be the fungus described by Petri isolated from chestnut trees." (*Blepharospora cambivora*.) This is undoubtedly a *Phytophthora*.

No. 1305 i, from lemon fruit, Corona, Calif.

No. 1306, from lemon gummosis.

No. 1308 f, from lemon fruit, Riverside, Calif.

No. 1309 a, from lemon gummosis, La Habra, Calif.

No. 1341, from orange fruit, Rivera, Calif.

The following organisms were sent by Tucker:

No. 1, from coconut bud; isolated by Tucker in Porto Rico.

No. 2, from cacao pod; isolated by Reinking in the Philippines; sent to Tucker by Ocfemia.

No. 3, from coconut bud; isolated by Ashby in Jamaica.

No. 4, from cacao pod; isolated by Ashby in Trinidad.

No. 6, from cacao pod; isolated by Roldan in the Philippines, sent to Tucker by Ocfemia.

No. 7, from cacao pod; isolated by Gadd in Ceylon.

No. 8, from cotton boll; isolated by Tucker in Porto Rico.

No. 9, from cotton boll; isolated by Ashby in St. Vincent.

No. 10, from cotton boll; isolated by Wakefield in Montserrat.

No. 11, from cotton boll; isolated by Ashby in Trinidad.

No. 12, from breadfruit; isolated by Gadd in Ceylon.

No. 13, from pawpaw fruit; isolated by Gadd in Ceylon.

No. 16, from *Dendrobium Maccarthiae*; isolated by Gadd in Ceylon.

No. 161, from eggplant; sent to Tucker by Dreschler who received it from Kendrick.

- No. 162, sent to Tucker by Dreschler, labeled "Church 528 *Phytophthora* Reddick II." Leonian's "Mutant" of Reddick's *Phytophthora*.
 No. 165, from Bermuda lily stump rot; sent to Tucker by Dreschler.
 No. 178, from grapefruit; sent to Tucker by Dreschler who received it from Fulton, originally labeled "Grapefruit *Phytoph.* P. R. 1."
 No. 181, from tomato fruit; sent to Tucker by Dreschler who received it from Kendrick, originally labeled "Kendrick 3269."
 No. 182, from eggplant; sent to Tucker by Dreschler who received it from Kendrick.
 No. 183, from pepper fruit; sent to Tucker by Dreschler who received it from Kendrick, originally labeled "Kendrick 3259."
 No. 185, from pineapple, Jamaica; sent to Tucker by Dreschler who received it from Hansford.
 No. 186, from tomato fruit; sent to Tucker by Dreschler who received it from Kendrick, originally labeled "Kendrick 3207."
 No. 187, from tomato buckeye rot, Washington, D. C.; sent to Tucker by Dreschler, originally labeled "*P. parasitica* var. *lycopersici*."
 No. 191, from tomato root; sent to Tucker by Dreschler.
 No. 194, from *Lilium candidum*; sent to Tucker by Dreschler.
 No. 197, from potato tuber, Kentucky; sent to Tucker by Dreschler who received it from Baarn.
 No. 201, from eggplant, Philippines; sent to Tucker by Dreschler who received it from Ocfemia; originally labeled "Eggplant *Phytophthora* II."
 No. 203, from potato tuber, Oklahoma; sent to Tucker by Dreschler.
 No. 206, from potato tuber, Idaho; sent to Tucker by Dreschler; labeled "Idaho 969."
 No. 207, from potato; sent to Tucker by Dreschler who received it from Welch labeled "*P. infestans*;" not *P. infestans* according to Dreschler; undoubtedly a strain of *P. omnivora*.
 No. 213, from eggplant in Philippines; sent to Tucker by Ocfemia; labeled "*P. melongenae*;" this is nothing but *P. omnivora*.

TECHNIC

The writers have followed the method developed by Leonian (13), which consists of obtaining a vigorously growing sterile mycelium and allowing it to fruit in a simple solution. The nutrient solution used in this work contains the following ingredients:

Nucleinic acid.....	1.0 gram.
Dihydrogen potassium phosphate.....	.6 gram.
Magnesium sulfate.....	.3 gram.
Dextrose.....	3.6 grams.
Distilled water.....	1,000 cubic centimeters.

Twenty-five cubic centimeters of this solution was poured into each of a series of glass capsules (preparation dishes) of 35 cubic centimeters capacity and sterilized. Then a bit of mycelium was transferred to this from the primary-stock cultures carried on oatmeal agar, and allowed to grow. Such colonies constituted the secondary stock cultures from which transfers were made to another series of capsules for the production of sterile mycelia. The cultures were then incubated at 25° C. until a good growth of submerged sterile mycelium was obtained in each dish, a period of three days usually being found sufficient. If allowed to remain longer, the hyphae may reach the surface of the solution and form zoosporangia. The sterile colonies thus obtained were removed from the nutrient solution, washed in sterile distilled water, and transferred to glass capsules each containing 2 cubic centimeters of M/100 solution of potassium nitrate. These cultures were then incubated for three days at 25°, and thereafter examined for zoosporangia. Since only reproduction

and no vegetative growth can take place in potassium nitrate solution, the number of immature sporangia was thus cut down to a minimum.

In most cases 200 representative zoosporangia were measured. Occasionally, when too wide a variation was manifested, as many as 400 measurements were made. On the other hand, when the zoosporangia were too scanty in number or when they exhibited an apparently close uniformity in size, only 100 sporangia were measured.

The sporangia of *Phytophthora infestans*, *P. phaseoli*, and *P. thalictri* were taken directly from their respective hosts and measured because of the difficulty of growing these three organisms on artificial media.

TABLE 2.—Length and width dimensions of the sporangia expressed in microns

Phytophthora organism	Length		Width	
	Mean	Mode	Mean	Mode
<i>P. arecae</i>	34.52±0.422	37.0	24.67±0.222	25.9
<i>P. cactorum</i> , 1.....	32.67±.443	33.3	27.49±.292	29.6
<i>P. cactorum</i> , 2.....	36.48±.451	33.3	31.82±.310	33.3
<i>P. cactorum</i> , 3.....	39.55±.648	37.0	28.89±.315	29.6
<i>P. capsici</i>	44.51±.838	40.7	23.89±.371	29.6
<i>P. citrophthora</i>	49.17±.860	51.8	30.96±.321	31.3
<i>P. fagi</i>	36.22±.532	37.0	28.97±.289	29.6
<i>P. infestans</i>	30.08±.382	27.7	18.90±.165	18.5
<i>P. phaseoli</i>	34.81±.270	37.0	20.09±.170	18.5
<i>P. mexicana</i>	50.20±.804	51.8	32.93±.379	29.6
<i>P. colocasiae</i>	37.70±.909	37.0	24.05±.404	25.9
<i>P. faberi</i>	39.66±.533	37.0	30.63±.331	29.6
<i>P. palmivora</i>	39.18±.490	37.0	25.67±.194	25.9
<i>P. parasitica</i>	40.03±.608	44.4	31.89±.419	33.3
<i>P. parasitica rhei</i> , 1.....	40.77±.610	40.7	30.11±.428	25.9
<i>P. parasitica rhei</i> , 11.....	40.14±.639	44.4	30.82±.424	33.3
<i>P. parasitica rhei</i> , IV.....	27.01±.332	24.5	25.93±.354	22.2
<i>P. sp.</i> (Reddick), 1.....	43.36±.978	37.0	30.52±.473	29.6
<i>P. sp.</i> (Reddick), 11.....	46.80±.788	48.1	36.03±.536	37.0
<i>P. terrestris</i>	41.03±.565	44.4	22.15±.366	37.0
<i>P. pini</i>	43.25±.518	37.0	28.78±.282	29.6
<i>P. thalictri</i>	20.60±.187	22.2	15.76±.168	14.8
<i>P. sp.</i> 8 (Hartley).....	33.52±.574	29.6	25.38±.287	25.9
<i>P. sp.</i> 22 (Hartley).....	42.62±.556	44.4	30.52±.361	29.6
<i>P. sp.</i> 26 (Hartley).....	38.62±.598	37.0	30.08±.412	29.6
<i>P. sp.</i> 36 (Hartley).....	36.03±.435	37.0	28.56±.318	29.6
<i>P. sp.</i> 44 (Hartley).....	35.06±.413	37.0	28.23±.307	25.9
<i>P. sp.</i> 97 (Hartley).....	40.81±.781	33.3	28.34±.285	25.9
<i>P. sp.</i> 100 (Hartley).....	35.06±.546	37.0	28.60±.282	29.6
<i>P. sp.</i> 102 (Hartley).....	38.40±.623	37.0	29.89±.319	29.6
<i>P. sp.</i> 116 (Hartley).....	41.81±.725	37.0	26.93±.271	25.9
<i>P. sp.</i> 117 (Hartley).....	41.84±.605	37.0	31.22±.348	33.3
<i>P. sp.</i> 121 (Hartley).....	40.03±.745	37.0	30.11±.295	29.6
<i>P. sp.</i> 123 (Hartley).....	40.75±.814	37.0	33.59±.512	33.3
<i>P. sp.</i> 126 (Hartley).....	36.29±.486	37.0	29.93±.322	25.9
<i>P. sp.</i> 130 (Hartley).....	37.29±.464	35.1	28.37±.282	29.6
<i>P. sp.</i> 136 (Hartley).....	37.44±.412	37.0	27.97±.235	29.6
<i>P. sp.</i> 137 (Hartley).....	36.26±.562	37.0	28.86±.329	29.6
<i>P. sp.</i> 138 (Hartley).....	44.51±.601	48.1	32.00±.359	37.0
<i>P. sp.</i> 140 (Hartley).....	39.70±.580	37.0	32.37±.443	29.6
<i>P. sp.</i> 141 (Hartley).....	36.77±.469	33.3	29.41±.443	29.6
<i>P. sp.</i> 142 (Hartley).....	36.88±.751	29.6	28.78±.336	29.6
<i>P. sp.</i> 143 (Hartley).....	33.44±.462	29.6	29.74±.340	29.6
<i>P. sp.</i> 144 (Hartley).....	32.59±.420	37.0	27.04±.299	29.6
<i>P. sp.</i> 145 (Hartley).....	44.06±1.402	33.3	30.30±.303	29.6
<i>P. sp.</i> (on Bryophilum).....	36.33±.663	33.3	30.26±.420	29.6
<i>P. sp.</i> (on tobacco).....	39.88±.611	33.3	30.45±.451	29.6
<i>P. sp.</i> (on tomato).....	31.22±.515	25.9	27.01±.363	25.9
<i>P. sp.</i> (on Vinca).....	38.03±.587	40.7	29.78±.389	29.6
<i>P. sp.</i> 760 (Fawcett).....	37.85±.551	37.0	30.19±.358	29.6
<i>P. sp.</i> 846 (Fawcett).....	47.36±.683	44.4	28.63±.214	29.6
<i>P. sp.</i> 1026 (Fawcett).....	43.30±.498	40.7	29.48±.265	29.6
<i>P. sp.</i> 1216 (Fawcett).....	34.85±.548	31.3	25.45±.341	25.9
<i>P. sp.</i> 1245 (Fawcett).....	45.17±.392	48.1	29.78±.230	29.6
<i>P. sp.</i> 1248 (Fawcett).....	38.07±.577	37.0	25.56±.264	25.9
<i>P. sp.</i> 1274 (Fawcett).....	41.10±.390	37.0	24.34±.191	25.9
<i>P. sp.</i> 1287 (Fawcett).....	51.54±.655	50.0	30.45±.252	29.6

TABLE 2.—Length and width dimensions of the sporangia expressed in microns—
Continued

Phytophthora organism	Length		Width	
	Mean	Mode	Mean	Mode
P. sp. 1290b (Fawcett)	49.54±1.140	37.0	30.81±.336	29.6
P. sp. 1290d (Fawcett)	37.44±.564	29.6	23.71±.367	22.2
P. sp. 1305i (Fawcett)	43.32±.539	44.4	30.15±.288	29.6
P. sp. 1306 (Fawcett)	52.45±1.381	44.4	30.56±.364	29.6
P. sp. 1308F (Fawcett)	46.32±.687	44.4	30.08±.295	29.6
P. sp. 1309a (Fawcett)	50.50±.909	48.1	28.67±.346	29.6
P. sp. 1341 (Fawcett)	49.35±.803	48.1	31.33±.359	33.3
P. sp. 1 (Tucker)	39.40±.475	37.0	28.23±.312	25.9
P. sp. 2 (Tucker)	35.92±.432	33.3	30.00±.317	29.6
P. sp. 3 (Tucker)	42.14±.541	44.4	27.15±.235	29.6
P. sp. 4 (Tucker)	38.85±.457	37.2	32.15±.361	29.6
P. sp. 6 (Tucker)	46.95±.802	48.1	31.74±.353	29.6
P. sp. 7 (Tucker)	40.62±.441	44.4	32.30±.353	33.3
P. sp. 8 (Tucker)	43.51±.561	46.2	36.70±.336	40.7
P. sp. 9 (Tucker)	48.35±.674	51.8	29.04±.296	29.6
P. sp. 10 (Tucker)	49.61±.589	48.1	40.44±.469	40.7
P. sp. 11 (Tucker)	48.65±.977	44.4	32.74±.281	29.6
P. sp. 12 (Tucker)	36.55±.348	37.0	28.12±.266	25.9
P. sp. 13 (Tucker)	39.50±.507	44.4	29.34±.333	33.3
P. sp. 16 (Tucker)	40.25±.518	37.0	28.93±.260	29.6
P. sp. 161 (Tucker)	39.92±.576	40.7	30.04±.410	29.6
P. sp. 165 (Tucker)	40.33±.606	33.3	33.33±.473	29.6
P. sp. 178 (Tucker)	50.13±.906	48.1	34.11±.458	29.6
P. sp. 181 (Tucker)	48.50±.876	40.7	32.89±.363	29.6
P. sp. 182 (Tucker)	40.99±.906	29.6	31.22±.452	29.6
P. sp. 183 (Tucker)	40.7±.807	37.0	30.93±.482	29.6
P. sp. 185 (Tucker)	43.10±.586	44.4	30.96±.330	33.3
P. sp. 186 (Tucker)	39.88±.587	40.7	31.70±.326	29.6
P. sp. 187 (Tucker)	41.88±.441	44.4	34.07±.371	37.0
P. sp. 191 (Tucker)	40.62±.766	33.3	32.56±.467	29.6
P. sp. 194 (Tucker)	32.85±.279	33.3	26.49±.265	25.9
P. sp. 197 (Tucker)	44.58±.661	48.1	32.39±.364	37.0
P. sp. 201 (Tucker)	38.99±.581	37.0	31.22±.386	29.6
P. sp. 203 (Tucker)	46.95±1.189	29.6	30.74±.489	25.9
P. sp. 206 (Tucker)	42.14±.420	38.8	27.13±.173	37.0
P. sp. 207 (Tucker)	44.95±.650	44.4	36.44±.379	37.0
P. sp. 213 (Tucker)	36.40±.382	37.0	28.30±.281	25.9

RESULTS

The results of the measurements made by the writers appear in Table 2. The authors have checked each other's measurements to make certain that no unconscious selection was practiced and that errors caused by the personal factor were reduced to a minimum. While the measurements checked fairly closely, it is recognized that the measurements of zoosporangia can never be highly standardized nor confined to the limits of mathematical pigeonholes.

The length of sporangia arranged according to the arbitrary size limits of 20–25 microns, 28–33 microns, 36–40 microns, 43–48 microns, and 50 microns and over, yields five groups, A, B, C, D, and E as shown in Table 3.

TABLE 3.—Grouping of *Phytophthoras* according to the mean length of sporangia

Group A, 20 to 25 microns	Group B, 28 to 33 microns	Group C, 36 to 40 microns	Group D, 43 to 48 microns	Group E, 50 microns and over
<i>P. thalietri</i> .	<i>P. arecae</i> . <i>P. cactorum</i> , I. <i>P. infestans</i> . <i>P. phascoli</i> . <i>P. parasitica rhei</i> , IV. <i>P. sp.</i> 8 (Hartley). <i>P. sp.</i> 143 (Hartley). <i>P. sp.</i> 144 (Hartley). <i>P. sp.</i> (tomato). <i>P. sp.</i> 1216 (Fawcett). <i>P. sp.</i> 194 (Tucker).	<i>P. cactorum</i> , 2. <i>P. cactorum</i> , 3. <i>P. fagi</i> . <i>P. colocasiae</i> . <i>P. faberi</i> . <i>P. palmivora</i> . <i>P. parasitica</i> . <i>P. parasitica rhei</i> , I. <i>P. parasitica rhei</i> , II. <i>P. terrestris</i> . <i>P. sp.</i> 26 (Hartley). <i>P. sp.</i> 36 (Hartley). <i>P. sp.</i> 44 (Hartley). <i>P. sp.</i> 97 (Hartley). <i>P. sp.</i> 100 (Hartley). <i>P. sp.</i> 102 (Hartley). <i>P. sp.</i> 121 (Hartley). <i>P. sp.</i> 123 (Hartley). <i>P. sp.</i> 126 (Hartley). <i>P. sp.</i> 130 (Hartley). <i>P. sp.</i> 136 (Hartley). <i>P. sp.</i> 137 (Hartley). <i>P. sp.</i> 139 (Hartley). <i>P. sp.</i> 140 (Hartley). <i>P. sp.</i> 141 (Hartley). <i>P. sp.</i> 142 (Hartley). <i>P. sp.</i> (Bryophilum). <i>P. sp.</i> (tobacco). <i>P. sp.</i> (Vina). <i>P. sp.</i> 760 (Fawcett). <i>P. sp.</i> 1246 (Fawcett). <i>P. sp.</i> 1274 (Fawcett). <i>P. sp.</i> 1290 d (Fawcett). <i>P. sp.</i> 1 (Tucker). <i>P. sp.</i> 2 (Tucker). <i>P. sp.</i> 4 (Tucker). <i>P. sp.</i> 7 (Tucker). <i>P. sp.</i> 12 (Tucker). <i>P. sp.</i> 13 (Tucker). <i>P. sp.</i> 16 (Tucker). <i>P. sp.</i> 161 (Tucker). <i>P. sp.</i> 165 (Tucker). <i>P. sp.</i> 182 (Tucker). <i>P. sp.</i> 183 (Tucker). <i>P. sp.</i> 186 (Tucker). <i>P. sp.</i> 191 (Tucker). <i>P. sp.</i> 201 (Tucker). <i>P. sp.</i> 213 (Tucker).	<i>P. capsici</i> . <i>P. citrophthora</i> . <i>P. sp.</i> (Reddick), I. <i>P. sp.</i> (Reddick), II. <i>P. pini</i> . <i>P. sp.</i> , 22 (Hartley). <i>P. sp.</i> 116 (Hartley). <i>P. sp.</i> 117 (Hartley). <i>P. sp.</i> 138 (Hartley). <i>P. sp.</i> 145 (Hartley). <i>P. sp.</i> 846 (Fawcett). <i>P. sp.</i> 1026 (Fawcett). <i>P. sp.</i> 1245 (Fawcett). <i>P. sp.</i> 1305 (Fawcett). <i>P. sp.</i> 1308 (Fawcett). <i>P. sp.</i> 3 (Tucker). <i>P. sp.</i> 6 (Tucker). <i>P. sp.</i> 8 (Tucker). <i>P. sp.</i> 9 (Tucker). <i>P. sp.</i> 10 (Tucker). <i>P. sp.</i> 11 (Tucker). <i>P. sp.</i> 181 (Tucker). <i>P. sp.</i> 185 (Tucker). <i>P. sp.</i> 187 (Tucker). <i>P. sp.</i> 197 (Tucker). <i>P. sp.</i> 203 (Tucker). <i>P. sp.</i> 206 (Tucker). <i>P. sp.</i> 207 (Tucker).	<i>P. mexicana</i> . <i>P. sp.</i> 1287 (Fawcett). <i>P. sp.</i> 1290 b (Fawcett). <i>P. sp.</i> 1306 (Fawcett). <i>P. sp.</i> 1309 (Fawcett). <i>P. sp.</i> 1341 (Fawcett). <i>P. sp.</i> 178 (Tucker).

The foregoing groups can by no means be considered stable, as Group A may easily merge with the lower limits of Group B, although not always so readily with those of C; similarly Group B may merge with the lower limits of C more frequently than with those of D, and so on. Group C, characterized by a size factor of 36-40 microns, contains the majority of individuals, followed by the next group which is distinguished by a length of 43-48 microns.

If grouped according to the width of sporangia, and according to the following arbitrary sizes of 18-22 microns, 25-28 microns, 31-34 microns, and 36 microns and over, four groups can be formed as shown in Table 4.

TABLE 4.—*Grouping of Phytophthoras according to the mean width of sporangia*

Group A, 18 to 22 microns	Group B, 25 to 28 microns	Group C, 31 to 34 microns	Group D, 36 microns and over
<i>P. infestans</i> . <i>P. phaseoli</i> . <i>P. thalictri</i> . <i>P. sp.</i> 1290d (Fawcett).	<i>P. arecae</i> . <i>P. cactorum</i> , 1. <i>P. cactorum</i> , 3. <i>P. capsici</i> . <i>P. fagi</i> . <i>P. colocasiae</i> . <i>P. palmivora</i> . <i>P. parasitica</i> rhei, IV. <i>P. pini</i> . <i>P. sp.</i> 8 (Hartley). <i>P. sp.</i> 36 (Hartley). <i>P. sp.</i> 44 (Hartley). <i>P. sp.</i> 97 (Hartley). <i>P. sp.</i> 100 (Hartley). <i>P. sp.</i> 102 (Hartley). <i>P. sp.</i> 116 (Hartley). <i>P. sp.</i> 126 (Hartley). <i>P. sp.</i> 130 (Hartley). <i>P. sp.</i> 136 (Hartley). <i>P. sp.</i> 137 (Hartley). <i>P. sp.</i> 141 (Hartley). <i>P. sp.</i> 142 (Hartley). <i>P. sp.</i> 143 (Bartley). <i>P. sp.</i> 144. <i>P. sp.</i> tomato. <i>P. sp.</i> (Vinea). <i>P. sp.</i> 846 (Fawcett). <i>P. sp.</i> 1026 (Fawcett). <i>P. sp.</i> 1216 (Fawcett). <i>P. sp.</i> 1245 (Fawcett). <i>P. sp.</i> 1246 (Fawcett). <i>P. sp.</i> 1274 (Fawcett). <i>P. sp.</i> 1302a (Fawcett). <i>P. sp.</i> 1 (Tucker). <i>P. sp.</i> 3 (Tucker). <i>P. sp.</i> 9 (Tucker). <i>P. sp.</i> 12 (Tucker). <i>P. sp.</i> 13 (Tucker). <i>P. sp.</i> 16 (Tucker). <i>P. sp.</i> 94 (Tucker). <i>P. sp.</i> 206 (Tucker). <i>P. sp.</i> 213 (Tucker).	<i>P. cactorum</i> 2. <i>P. citrophthora</i> . <i>P. mexicana</i> . <i>P. faberi</i> . <i>P. parasitica</i> . <i>P. parasitica</i> rhei, I. <i>P. parasitica</i> rhei, II. <i>P. sp.</i> (Reddick). <i>P. terrestris</i> . <i>P. sp.</i> 22 (Hartley). <i>P. sp.</i> 26 (Hartley). <i>P. sp.</i> 117 (Hartley). <i>P. sp.</i> 121 (Hartley). <i>P. sp.</i> 123 (Hartley). <i>P. sp.</i> 138 (Hartley). <i>P. sp.</i> 139 (Hartley). <i>P. sp.</i> 140 (Hartley). <i>P. sp.</i> 145 (Hartley). <i>P. sp.</i> (Bryophilum). <i>P. sp.</i> (tobacco). <i>P. sp.</i> 760 (Fawcett). <i>P. sp.</i> 1287 (Fawcett). <i>P. sp.</i> 1290b (Fawcett). <i>P. sp.</i> 1305b (Fawcett). <i>P. sp.</i> 1306b (Fawcett). <i>P. sp.</i> 1308b (Fawcett). <i>P. sp.</i> 1341b (Fawcett). <i>P. sp.</i> 2 (Tucker). <i>P. sp.</i> 4 (Tucker). <i>P. sp.</i> 6 (Tucker). <i>P. sp.</i> 7 (Tucker). <i>P. sp.</i> 8 (Tucker). <i>P. sp.</i> 10 (Tucker). <i>P. sp.</i> 11 (Tucker). <i>P. sp.</i> 161 (Tucker). <i>P. sp.</i> 162 (Tucker). <i>P. sp.</i> 165 (Tucker). <i>P. sp.</i> 178 (Tucker). <i>P. sp.</i> 181 (Tucker). <i>P. sp.</i> 182 (Tucker). <i>P. sp.</i> 183 (Tucker). <i>P. sp.</i> 185 (Tucker). <i>P. sp.</i> 186 (Tucker). <i>P. sp.</i> 187 (Tucker). <i>P. sp.</i> 191 (Tucker). <i>P. sp.</i> 197 (Tucker). <i>P. sp.</i> 201 (Tucker). <i>P. sp.</i> 203 (Tucker). <i>P. sp.</i> 207 (Tucker).	<i>P. sp.</i> 8 (Tucker). <i>P. sp.</i> 10 (Tucker). <i>P. sp.</i> 162 (Tucker). <i>P. sp.</i> 207 (Tucker).

The apparent or real kinship of the various organisms becomes still more pronounced where individual strains possessing identical lengths or widths are grouped together as in Table 5.

TABLE 5.—*Grouping together of Phytophthoras with identical length and width of sporangia*

Length	Organism	Width	Organism
<i>Microns</i>		<i>Microns</i>	
20	<i>P. thalictri</i> .	15	<i>P. thalictri</i> .
27	<i>P. parasitica</i> rhei, IV.	18	<i>P. infestans</i> .
30	<i>P. infestans</i> .	20	<i>P. phaseoli</i> .
31	<i>P. sp.</i> (tomato).	23	<i>P. sp.</i> 1290d (Fawcett).
32	<i>P. cactorum</i> , 1.	24	<i>P. arecae</i> .
	<i>P. sp.</i> 144 (Hartley).		<i>P. colocasiae</i> .
	<i>P. sp.</i> 194 (Tucker).		<i>P. sp.</i> 1274 (Fawcett).

TABLE 5.—Grouping together of *Phytophthoras* with identical length and width of sporangia—Continued

Length	Organism	Width	Organism
<i>Microns</i>		<i>Microns</i>	
33	P. sp. 8 (Hartley).	25	P. parasitica rhei, IV.
	P. sp. 143 (Hartley).		P. sp. 8 (Hartley).
	P. arecae.		P. sp. 1216 (Fawcett).
34	P. phaseoli.		P. sp. 1246 (Fawcett).
	P. sp. 1216 (Fawcett).		P. palmivora.
	P. sp. 44 (Hartley).	26	P. sp. 116 (Hartley).
35	P. sp. 100 (Hartley).		P. sp. 194 (Tucker).
	P. sp. 2 (Tucker).		P. cactorum, 1.
	P. cactorum 2.		P. sp. 136 (Hartley).
	P. fagi.		P. sp. 144 (Hartley).
	P. sp. 36 (Hartley).	27	P. sp. (tomato).
36	P. sp. 126 (Hartley).		P. sp. 3 (Tucker).
	P. sp. 137 (Hartley).		P. sp. 206 (Tucker).
	P. sp. 141 (Hartley).		P. cactorum, 3.
	P. sp. 142 (Hartley).		P. fagi.
	P. colocasiae.		P. pini.
	P. sp. 130 (Hartley).		P. sp. 36 (Hartley).
	P. sp. 136 (Hartley).		P. sp. 44 (Hartley).
37	P. sp. 760 (Fawcett).		P. sp. 97 (Hartley).
	P. sp. 1290d (Fawcett).		P. sp. 100 (Hartley).
	P. sp. 4 (Tucker).	28	P. sp. 130 (Hartley).
	P. sp. 201 (Tucker).		P. sp. 137 (Hartley).
	P. sp. 26 (Hartley).		P. sp. 142 (Hartley).
	P. sp. 102 (Hartley).		P. sp. 846 (Fawcett).
38	P. sp. (Vinea).		P. sp. 1309 (Fawcett).
	P. sp. 1246 (Fawcett).		P. sp. 1 (Tucker).
	P. sp. 4 (Tucker).		P. sp. 12 (Tucker).
	P. sp. 201 (Tucker).		P. sp. 16 (Tucker).
	P. cactorum, 3.		P. sp. 213 (Tucker).
	P. faberi.		P. capsici.
	P. palmivora.		P. sp. 102 (Hartley).
39	P. sp. 139 (Hartley).	29	P. sp. 126 (Hartley).
	P. sp. 140 (Hartley).		P. sp. 141 (Hartley).
	P. sp. (tobacco).		P. sp. 143 (Hartley).
	P. sp. 1 (Tucker).		P. sp. (Vinea).
	P. sp. 13 (Tucker).		P. sp. 1026 (Fawcett).
	P. sp. 161 (Tucker).		P. sp. 1245 (Fawcett).
	P. sp. 186 (Tucker).		P. sp. 9 (Tucker).
	P. parasitica.		P. sp. 15 (Tucker).
	P. parasitica rhei, I.		P. citrophthora.
	P. parasitica rhei, II.		P. faberi.
	P. sp. 97 (Hartley).		P. parasitica rhei, I.
40	P. sp. 121 (Hartley).		P. parasitica rhei, II.
	P. sp. 123 (Hartley).		P. sp. (Reddick), I.
	P. sp. 7 (Tucker).		P. sp. 22 (Hartley).
	P. sp. 16 (Tucker).		P. sp. 26 (Hartley).
	P. sp. 165 (Tucker).		P. sp. 121 (Hartley).
	P. sp. 182 (Tucker).		P. sp. 145 (Hartley).
	P. sp. 191 (Tucker).		P. sp. (Bryophilum).
	P. terrestris.	30	P. sp. (Tobacco).
	P. sp. 116 (Hartley).		P. sp. 760 (Fawcett).
41	P. sp. 117 (Hartley).		P. sp. 1287 (Fawcett).
	P. sp. 1274 (Hartley).		P. sp. 1290b (Fawcett).
	P. sp. 187 (Tucker).		P. sp. 1305 (Fawcett).
	P. sp. (Reddick) I.		P. sp. 1306 (Fawcett).
	P. pini.		P. sp. 1308 (Fawcett).
43	P. sp. 1026 (Fawcett).		P. sp. 2 (Tucker).
	P. sp. 1305 (Fawcett).		P. sp. 161 (Tucker).
	P. sp. 8 (Tucker).		P. sp. 183 (Tucker).
	P. sp. 185 (Tucker).		P. sp. 185 (Tucker).
	P. capsici.		P. sp. 203 (Tucker).
	P. sp. 138 (Hartley).		P. cactorum 2.
44	P. sp. 145 (Hartley).		P. parasitica.
	P. sp. 197 (Tucker).		P. sp. 117 (Hartley).
	P. sp. 207 (Tucker).		P. sp. 139 (Hartley).
45	P. sp. 1245 (Fawcett).	31	P. sp. 1341 (Fawcett).
	P. sp. (Reddick) II.		P. sp. 6 (Tucker).
46	P. sp. 1308 (Fawcett).		P. sp. 182 (Tucker).
	P. sp. 6 (Tucker).		P. sp. 186 (Tucker).
	P. sp. 203 (Tucker).		P. sp. 201 (Tucker).

TABLE 5.—Grouping together of *Phytophthoras* with identical length and width of sporangia—Continued

Length	Organism	Width	Organism
<i>Microns</i>		<i>Microns</i>	
47	<i>P. sp.</i> 846 (Fawcett).		<i>P. mexicana</i> .
	<i>P. sp.</i> 9 (Tucker).		<i>P. terrestris</i> .
48	<i>P. sp.</i> 11 (Tucker).		<i>P. sp.</i> 132 (Hartley).
	<i>P. sp.</i> 181 (Tucker).		<i>P. sp.</i> 140 (Hartley).
	<i>P. citrophthora</i> .		<i>P. sp.</i> 1 (Tucker).
49	<i>P. sp.</i> 1290b (Fawcett).	32	<i>P. sp.</i> 4 (Tucker).
	<i>P. sp.</i> 1341 (Fawcett).		<i>P. sp.</i> 7 (Tucker).
	<i>P. sp.</i> 10 (Tucker).		<i>P. sp.</i> 181 (Tucker).
	<i>P. mexicana</i> .		<i>P. sp.</i> 191 (Tucker).
50	<i>P. sp.</i> 1309a (Fawcett).		<i>P. sp.</i> 197 (Tucker).
	<i>P. sp.</i> 178 (Tucker).	33	<i>P. sp.</i> 123 (Hartley).
51	<i>P. sp.</i> 1287 (Fawcett).		<i>P. sp.</i> 165 (Tucker).
52	<i>P. sp.</i> 1306 (Fawcett).	34	<i>P. sp.</i> 178 (Tucker).
			<i>P. sp.</i> 187 (Tucker).
			<i>P. sp.</i> 8 (Tucker).
		36	<i>P. sp.</i> 207 (Tucker).
			<i>P. sp.</i> (Reddick) II.
		40	<i>P. sp.</i> 10 (Tucker).

DISCUSSION OF RESULTS

It is very evident that similarity or dissimilarity of sporangial sizes does not, necessarily, denote taxonomic kinship. Whereas in reality *Phytophthora thalictri* should be nothing more than a variety of *P. infestans*, it was found that from the standpoint of sporangial sizes alone it is much closer to *P. parasitica* var. *rhei* IV. *P. arecae* and *P. phaseoli* are decidedly different species, yet they are almost identical in so far as the size of their zoosporangia is concerned. There are 28 organisms (Table 5) between *P. cactorum* 1 and *P. cactorum* 3 if the length of sporangia is considered, and only 8 organisms between *P. cactorum* 1 and *P. cactorum* 2, whereas, from the standpoint of the width of zoosporangia, it stands next to *P. cactorum* 3 and is 48 organisms removed from *P. cactorum* 2. If three strains of this one species alone show such sharp dissimilarities in the size of their sporangia, despite the rigidly controlled environmental conditions, it is natural that glaring discrepancies are to be noted throughout the taxonomic literature on this and other species of *Phytophthoras*. Similarly, if some of the strains of *P. parasitica rhei* which were dissociated under the very eye of the senior writer, exceed all reasonable limits of fluctuation and yield decidedly different looking individuals, it is not surprising that so many of the different strains of *P. omnivora* have been described as different species. Arranged according to the mean size of their zoosporangia the following 15 strains of *P. omnivora* manifest a remarkable gradation with a maximum difference of 22 microns between the mean length of *P. parasitica rhei* IV and *P. sp.* 10 (Tucker), and a difference of 15 microns between their mean width, not to say anything about all kinds of intergradations:

	<i>Microns</i>		<i>Microns</i>
<i>P. parasitica rhei</i> IV-----	27×25	<i>P. parasitica</i> (Dastur's strain) -	40×31
<i>P. sp.</i> (tomato)-----	31×27	<i>P. terrestris</i> -----	41×31
<i>P. sp.</i> 144-----	32×27	<i>P. sp.</i> (Reddick) I-----	43×30
<i>P. sp.</i> 143-----	33×29	<i>P. sp.</i> 145-----	44×30
<i>P. sp.</i> 126-----	36×29	<i>P. sp.</i> 138-----	44×32
<i>P. sp.</i> (Vinca)-----	38×29	<i>P. sp.</i> (Reddick) II-----	46×36
<i>P. sp.</i> 139-----	39×31	<i>P. sp.</i> 10 (Tucker)-----	49×40
<i>P. parasitica rhei</i> I-----	40×30		

Confronted by such a situation, and with no aid forthcoming from the available taxonomic literature, the worker is obliged to create many new species and varieties in order properly to dispose of such organisms. Yet the anomaly of such a procedure is at once apparent, because the writers know that at least in some cases the so-called new forms sprang from the old as dissociative phases and represent nothing but a stage in the life of a given organism rather than true mutants or immutable entities.

The size of oogonia or oospores is no more dependable for purposes of classification than that of sporangia. The following figures (in microns) for the oogonia of *Phytophthora cactorum*, as given by different authorities, speak for themselves:

16×24, DeBary	27.36, Rosenbaum, for <i>Panax</i> strain.
22×32, Stevens and Plunkett.	26 to 28, Hori.
26.78, Rosenbaum, for <i>Phyllocactus</i> strain.	24 to 30, Schroeter.
	20 to 70, Lebert and Cohn.

Because the two strains of *Phytophthora cactorum* which Rosenbaum studied averaged about 27 microns for the oospores one is not justified in assuming that the measurements of other workers were faulty. While it is true that the scheme of computing measurements and the environmental conditions utilized by any given investigator unquestionably exert a material influence upon the size factor, the innate variability and the specific or hereditary peculiarities of different strains may also be responsible for the obvious discrepancies noted in the foregoing figures. Even if 27 microns are accepted as representing the average size of the oospores of *P. cactorum*, one is still confronted by the existence of numerous other species the sexually formed spores of which measure not so very differently from those of *P. cactorum*. Consider, for example, the following measurements in microns:

<i>P. colocasiae</i>	20 to 28.	<i>P. phaseoli</i>	25.
<i>P. erythrosepica</i> ..	29 to 30 (MacMillan).	<i>P. syringae</i>	29.5 (Rosenbaum).
<i>P. erythrosepica</i> ..	35.78 (Rosenbaum).	<i>P. syringae</i>	25 (Lafferty and Pethybridge).
<i>P. fagi</i>	20 to 30.	<i>P. theobromae</i> ...	22 to 45.
<i>P. omnivora</i>	24 to 30.	<i>P. hibernalis</i> ...	22 to 45.
<i>P. parasitica rhei</i> ..	24.	<i>P. meadii</i>	16 to 32.

The differences which seem to exist in the size of oospores of different organisms are not large enough for such a fluctuating group of fungi to justify their employment in the primary differentiation of species. When a difference of 5 microns exists in the measurements of Lafferty and Pethybridge and those of Rosenbaum as given in the case of *Phytophthora erythrosepica*, it becomes rather difficult to accept a difference of 2 or 3 microns as of sufficient significance to be employed in taxonomy. Furthermore, since no uniformity of environmental conditions can be found described throughout the literature concerning oospore production and measurements, these figures, as they now exist, possess no value, and merely create confusion. Before the size of oospores can have any value for classification, all work must be repeated under standardized conditions, and at least 20 different strains of each species of *Phytophthora* should be used before figures of comparative reliance can be obtained.

Furthermore the relation of antheridia to oogonia is not of much significance taxonomically. There are at least four species, *Phytophthora cactorum*, *P. fagi*, *P. syringae*, and *P. pini* in which both para-

gynous and amphigynous antheridia are present. It is highly probable that additional species, if studied intensively enough, may show similar relationships.

The presence or absence of the sexual bodies is not a safe criterion in taxonomy. Aside from the possible occurrence of heterothallism, which has already been demonstrated in some strains of *Phytophthora faberi*, there is the further probability of cyclogenic successions in the life of a given fungus, whereby sexual and sexless phases may alternate irrespective of environmental conditions. Such instances are by no means rare in some strains of *P. infestans* where a fairly large number of oogonia may sometimes develop in oatmeal-agar tubes, and then, upon subsequent transfers, such bodies may altogether disappear for months at a time, no matter how often nor under what conditions subcultures are made. Then, just as suddenly, these bodies may reappear. *P. thalictri* is described by Wilson as possessing no oogonia; Davis failed to find such bodies; Clinton discovered very few in the specimens collected by him; but the strain which was collected in West Virginia and put in pure culture by Berg, forms oogonia in great abundance. Some strains of other species may form sexual bodies very readily, while others may not yield a single oogonium; or they may yield oogonia in culture for a while, and then cease producing them altogether. The senior writer has shown that the so-called mutants developing from a monosporangious culture of *P. parasitica rhei*, manifested some sharp differences in their ability to form sexual bodies. Strains I and II produced oogonia abundantly, Strain V less readily, Strain IV sparingly, and Strain III none at all. Yet these five strains were not mutants, although at first presented as such, but merely different phases in the cyclogeny of the same organism; after breeding true for over three years they reverted to their prototype, and all efforts again to induce segregation met with failure.²

Dissociation among fungi is of much greater significance than has hitherto been realized. Many, if not all, of the so-called mutants, saltants, variants, etc., are probably nothing more than dissociative phases in the life of the same fungus. It is difficult to conceive of an organism which will permanently lose or gain certain characteristics, as the adherents of the mutation theory would lead us to believe. If the environment can not put something in the protoplasm which was not there before, how can the same environment remove something that was there? All that environment can do is to cause the emergence or submergence of certain characters. Such being the case, it would be unwise to accept the assumption that the so-called new strains produced by the operation of the mutation phenomenon are specific organisms rather than merely so many phases or facets of one complex organisms. It has been suggested that because the media employed by the senior writer have produced so many dissociations among *Phytophthoras* and *Fusaria*, the employment of substrata rich in sugars and organic nitrogen should be avoided. But would not this merely be ignoring truth simply because it happened adversely to affect the smooth working of our taxonomic mechanism?

² Just before this paper went to the printer, some further work was undertaken with *P. parasitica rhei* and it was found that after a quiescent period of more than two years this organism began to dissociate once more, and that whereas it formerly produced only 5 strains, now there are nearly 20 of them, with no stability of growth characters in sight. It is probable that additional strains may appear and be isolated in the near future.

STATUS OF DIFFERENT SPECIES

In the light of the foregoing tables and discussions, what can be the status of different species of *Phytophthora*? While the writers have not had the opportunity of studying a large number of strains of all the species of this genus, and consequently feel rather reluctant to make too many decisive statements, it does seem that at least some of the species are well enough established to withstand future taxonomic upheavals. Others, however, have very little or no secure foundation and should be merged with the older species. The *infestans* group is decidedly secure with its 3 species, *P. infestans*, *P. thalictri*, and *P. phaseoli*. These 3 organisms are very readily distinguished from all others, not only by their morphological features, but especially by the difficulty with which they grow on synthetic media. Whereas all other forms of *Phytophthoras* studied by the writers make fairly large colonies on malt-extract agar within six days, these 3 species show not the slightest growth in that time. Their host relationship is very narrow and specialized, and their sporangia are among the smallest. Arranged according to the size of these bodies, from the smallest to the largest, these 3 species may be recognized in the following order: *P. thalictri*, *P. infestans*, and *P. phaseoli*. The next group which may be readily identified consists of *P. cinnamomi*, *P. cryptogea*, *P. erythroseptica*, and *P. mexicana*. The writers have not had the opportunity of studying many different strains of these organisms because no such strains were available, yet so far as their experience is concerned, at least 3 of these 4 species should stand for the time being. All 4 of them are scanty sporangia formers under most conditions; *P. cryptogea* has formed no such bodies in all the solutions employed by the senior writer; *P. cinnamomi* is the only one of the 4 capable of producing chlamydospores in solutions; in fact, it has formed only chlamydospores. *P. erythroseptica* and *P. mexicana* differ from the foregoing 2 by the absence of the chlamydospore-forming habit and by their ability to yield sporangia in dilute solutions of nitrates. The chief difference between *P. erythroseptica* and *P. mexicana* lies in the shape of their papillae, but this is a rather dubious distinction, and one that is extremely variable and highly influenced by the environmental conditions. The sporangia of *P. mexicana* may be somewhat larger, but the size factor can not be considered too seriously in making specific distinctions. It would simplify matters if *P. mexicana* were merged with *P. erythroseptica*, and there seems to be no valid reason why this should not be done.

Phytophthora capsici and *P. citrophthora* differ chiefly in the absence of sexual bodies from the latter species. But since there are strains of *P. infestans*, *P. thalictri*, *P. parasitica*, *P. faberi*, etc., described as possessing no sexual bodies, there is no reason why *P. capsici* should not be merged with *P. citrophthora*. The latter organism does not possess sufficiently distinctive specific characters to be very secure anyway, and it is probable that sooner or later it will be found necessary to classify it with the *omnivora* group. The same appears also to apply to *P. nicotianae*, but nothing definite can be said about this until more strains are available for study.

The only way *Phytophthora cactorum* can be distinguished from the *omnivora* group is by means of the position of the antheridia upon the oogonia. Yet, since this does not seem always to occur, and both

paragynous and amphigynous types of antheridia are known to form in *P. cactorum*, this species can be brought still closer to *P. omnivora*. Forms of *P. cactorum* which possess no sexual stage can be placed nowhere else except in *P. omnivora*, and if this species is retained, there can be no doubt that certain of its strains will be classified as *P. cactorum* and others as *P. omnivora*. However, a definite judgment should be suspended on this species, pending the completion of some work now in progress. That *P. fagi* can have no specific standing is reiterated here. The slight differences in the size of sporangia which exist between *P. cactorum* and *P. fagi*, and which have hitherto been considered of sufficient specific value, are no longer tenable because we have seen that while the mean length of *P. cactorum* 1 is 32.67 microns and that of *P. fagi* is 36.22 microns, *P. cactorum* 2 measures 36.48 microns and *P. cactorum* 3 goes up to 39.55 microns. Nor are the differences in the comparative sizes of oogonia large enough to be significant. The pathological relationships are just as unreliable: the numerous physiological races of various fungi and the complex and conflicting relationship of these races to their host plants do not permit an unrestricted use of pathogenicity in taxonomy.

Perhaps the most cosmopolitan and the most common of all *Phytophthoras* are those that have been gathered by the senior writer into the *omnivora* group. Extremely variable and of wide adaptability, these organisms have been a prolific source of confusion. *P. colocasiae*, *P. faberi*, *P. palmivora*, *P. parasitica*, *P. terrestris*, *P. parasitica rhei*, *P. melongenae*, and perhaps *P. theobromae*, *P. hibernalis*, *P. meadii*, *P. jatrophae*, *P. allii*, and all other so-called new species described from Japan and India and many of them not available in pure-culture form, belong here. The chief reform, therefore, should begin with this group. If any of the foregoing organisms described as distinct species possessed recognizable morphological or physiological characters, there would be no need of a reform. As it is, one may call a certain *Phytophthora* isolated from coconut or palm as *P. faberi* or *P. palmivora*, but the same culture mixed with a number of other strains of the *omnivora* group can not again be singled out with any degree of certainty. The five colonies of the assumed mutants of *P. parasitica rhei*, as illustrated by the senior writer (13), show a wide degree of deviation from the more common type of *P. parasitica* growth. Every macroscopic and microscopic character of this fungus seems to be modified in these five dissociative phases. The typical growth type of *P. parasitica* is a colony of comparatively slow growth, of curly, coagulated patterns, and of bulbous, knotted, variously enlarged and gnarled submerged hyphae. *P. parasitica* I and II illustrate this habit. The *P. faberi*, *P. palmivora* and *P. colocasiae* type of growth consists of a smooth-combed nature with even and regular submerged hyphae. *P. parasitica rhei* V illustrates this mode of growth, while III and IV show other intermediate stages.

The confusion which now exists in *Phytophthora omnivora* can be largely eliminated if we divide this group into six arbitrary types: 1, *colocasiae-microsporous*; 2, *colocasiae macrosporous*; 3, *faberi-microsporous*; 4, *faberi-macrosporous*; 5, *parasitica-microsporous*; and 6, *parasitica-macrosporous*. The first can be characterized by a smooth-combed colony habit and more elongated but smaller-sized

sporangia; the second is like the first except that the spores are larger; the third has the same colony habit but its sporangia are more spherical; the fourth is like the third, except that the sporangia are larger; the fifth type is characterized by curly, coagulated type of growth and gnarled submerged hyphae, with sporangia either elongated or spherical; the sixth type is like the fifth, except that the sporangia are larger. Such an arrangement provides for most, if not all, of the variations of this unstable group and would save the worker the anomaly of classifying the same organism under two or more specific names whenever such a fungus happens to dissociate and throw off dissimilar phases. No matter which dissociative stage or which type of strain happened to be isolated by a given worker, it could immediately be classified under a scheme which recognizes that a given culture does not always appear and behave the same way; that it may go through a more or less complicated cyclogeny; that dissociation of a fungus into its different phases can not be indefinitely controlled, and that striking deviations from the common specific path may often occur for various lengths of time. But because these are passive stages they should not be given any permanence by building a true taxonomic pedestal under them.

Blepharospora cambivora should be *Phytophthora cambivora*, and, as such, it may have a comparatively sound taxonomic basis. The lack of papillae on the sporangia of this species is largely a relative matter largely controlled by the environment. When, for instance, this organism was inoculated into green pepper fruits, many of the ensuing sporangia developed well-pronounced papillae, although blunt sporangia were also present in no negligible numbers. But since, under most conditions, the sporangia of *P. cambivora* remain characteristically blunt, this fungus should be considered sufficiently distinctive to be regarded as a new species of *Phytophthora*.

Phytophthora pini should be retained, at least for the time being. Of all the *Pythophthoras* grown by the writers this is the only one that forms both sporangia and oogonia in various solutions, a rather unique character, and, like the chlamydospore-forming habit of *P. cinnamomi*, unquestionably distinctive.

Hartley's organisms Nos. 8, 36 and 121 are sufficiently different from all others to merit further study in their native habitat to determine whether they should be described as new species.

SUMMARY

An attempt is made in this paper to compute the comparative value of the size of sporangia obtained under controlled conditions. Measurements of sporangia made by a number of representative workers are tabulated to show the discrepancies which exist in the literature due to the unstandardized methods of investigations, and to the great variability of the individual species. Because even the different strains of the same species vary so much that their sporangia cover the entire scale of sizes known in the reproductive bodies of *Phytophthoras*, the size factor can not be of primary importance in taxonomy. Oogonia and oospores show similar size variations and are therefore no more valuable in this respect.

Phytophthora infestans, *P. thalictri*, and *P. phaseoli* are taxonomically secure. *P. cinnamomi*, *P. cryptogea*, *P. erythrosetpica*, and

P. pini are also distinctive enough to be retained. *P. mexicana* should be merged with *P. erythroseptica* and *P. capsici* with *P. citrophthora*. *Blepharospora cambivora* should be *Phytophthora cambivora*, and *P. fagi* is the same as *P. cactorum*. *P. cactorum* is retained with reservations. *P. arecae*, *P. nicotianae*, and *P. syringae* must be considered of doubtful specific value as classifications until intensive study of a number of strains of these organisms has been made. *P. colocasiae*, *P. faberi*, *P. palmivora*, *P. parasitica*, *P. terrestris*, *P. parasitica rhei*, and *P. melongenae* should be merged with *P. omnivora*; it is probable that *P. theobromae*, *P. allii*, *P. jatrophae*, *P. meadii*, and *P. hibernalis* also belong in that group. Hartley's organisms Nos. 8, 36, and 121 seem to be sufficiently distinctive to merit further study.

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INFLUENCE OF VARIETAL RESISTANCE, SAP ACIDITY, AND CERTAIN ENVIRONMENTAL FACTORS ON THE OCCURRENCE OF LOOSE SMUT IN WHEAT¹

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INTRODUCTION

During the 10-year period 1917-1926, for which records are available,³ loose smut (*Ustilago tritici* (Pers.) Jens.) in the United States took an estimated toll of wheat which averaged more than 10,000,000 bushels annually. (Fig. 1.) Measures for controlling the disease in this country, however, rarely are applied. The very close association of the host and parasite throughout their life cycles makes practical control a difficult matter. The modified hot-water treatment of the seed, which has been generally recommended during the last 20 years and which has proved to be the best treatment yet devised, is effective in control. However, it involves a number of operations which make it tedious and difficult to apply under usual farm conditions.⁴ Moreover, the treatment retards emergence of

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² The writer wishes to express his thanks to H. H. Whetzel, professor of plant pathology at Cornell University, for helpful suggestions; to H. H. Love, professor of plant breeding at Cornell University, for providing facilities for carrying out the experiments in 1924-25; and to C. E. Leighty, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for supplying most of the seed samples used in the experiments and for assistance in identifying varieties.

³ UNITED STATES DEPARTMENT OF AGRICULTURE. BUREAU OF PLANT INDUSTRY. The following mimeographed material from Plant Disease Bulletin (or Reporter):

ESTIMATE OF CROP LOSSES DUE TO PLANT DISEASES. 1917. Plant Disease Bul. 2(1), 18 p. 1918. CROP LOSSES FROM PLANT DISEASES. 1918. Sup. 6, p. 186-213. 1919. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1919. Sup. 12, p. 307-332. 1920. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1920. Sup. 18, p. 317-338. 1921. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1921. Sup. 24, p. 489-510. 1922. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1922. Plant Disease Rptr., Sup. 30, p. 462-490. 1923. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1923. Sup. 36, p. 318-348. 1924. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1924. Sup. 43, p. 381-410. 1925. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1925. Sup. 49, p. 382-412. 1926. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1926. Sup. 56, p. 394-423. 1927. Also the following mimeographed supplements of the Plant Disease Bulletin (or Reporter): FROMME, F. D. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1920. Sup. 15, p. 115-176, illus. 1921. HASKELL, R. J. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1923. Sup. 35, p. 244-348, illus. 1924. ——— DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1925. Sup. 48, p. 301-381, illus. 1926. ——— DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1927. Sup. 62, p. 302-353. 1928. ——— and WOOD, J. I. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1922. Sup. 27, p. 164-266, illus. 1923. KIRBY, R. S., and ARCHER, W. A. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1926. Sup. 53, p. 110-208, illus. 1927. MELCHERS, L. R. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1924. Sup. 40, p. 106-191, illus. 1925. STAKMAN, E. C. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1921. Sup. 21, p. 139-254, illus. 1922.

⁴ These operations are: A 4 to 6 hour presoaking of the seed in cold water, a momentary tempering dip at about 120° F., a 10-minute bath at about 129°, sowing of the treated seed in a separate plot, and, finally, separate harvesting of the plants from treated seed.

seedlings and generally reduces germination and yield (34, 35).⁵ The inconspicuous nature of the loss caused by loose smut in wheat doubtless also helps to account for the lack of an active practicing

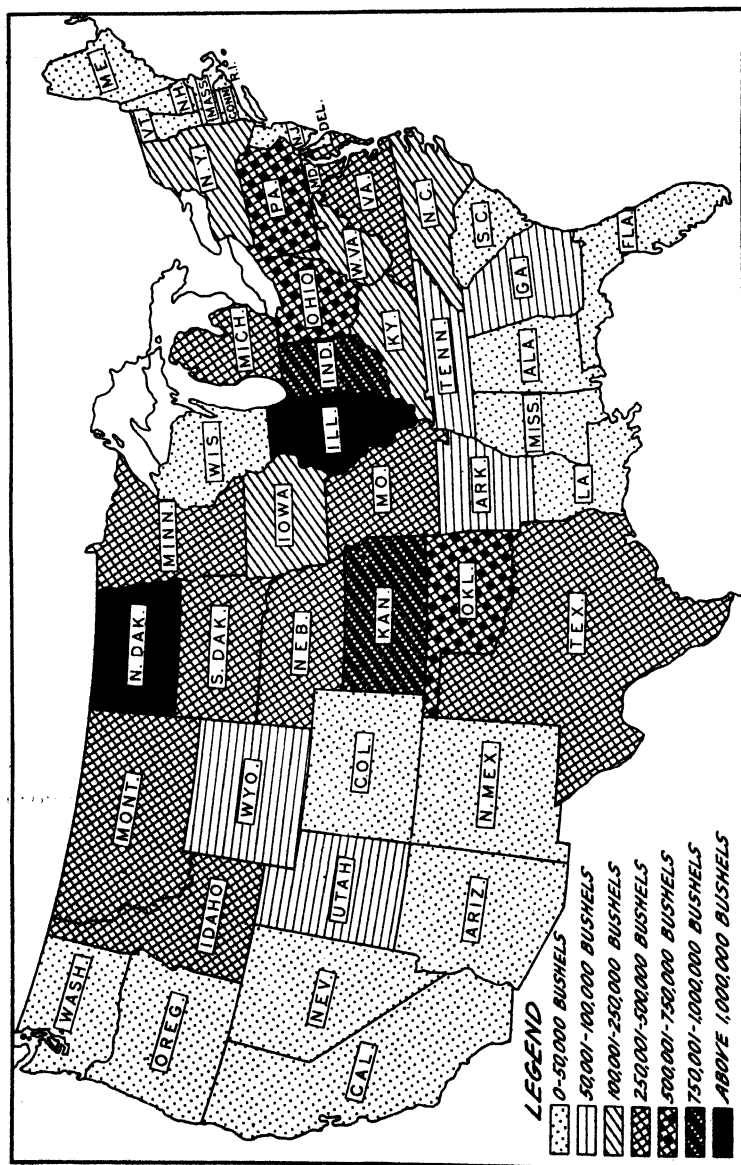


FIGURE 1.—Estimated average annual reduction of wheat production (bushels) due to loose smut in each State for the period 1917-1926, inclusive. The average reduction in bushels for each State has been placed in one of seven groups, as shown in the legend. Data compiled from estimates of the Plant Disease Bulletin (footnote 3)

of control. The smutted heads, the only signs of the disease that are readily apparent, are in evidence for a few days only, i. e., during the blooming period of the healthy heads. As a result, growers

⁵ Reference is made by number (italic) to "Literature cited," p. 337.

frequently are led to underestimate or to overlook entirely the amount of grain destroyed and the need for control. Moreover, loose smut in wheat never becomes alarmingly severe except in individual fields or within limited territory. Five per cent of diseased heads seems to be the highest amount that has been recorded for state-wide areas.⁶ It is difficult, therefore, to enlist a general interest in control among growers, especially in view of the fact that all the known control measures are relatively difficult of application.

In consideration of these factors it would seem that the control of loose smut in wheat presents a problem particularly adapted for solution through the selection or development of resistant varieties, or of resistant strains within varieties which are susceptible but nevertheless desirable. To this end the studies on varietal resistance reported herein were undertaken with a number of wheats mostly from the eastern half of the United States. There are reported herein also the results of studies to determine (1) a possible correlation between high acidity in the cell sap of wheat of different varieties and their resistance to loose smut, and (2) the extent to which certain environmental factors may influence the amount of disease exhibited by a particular variety. Several previous investigations, as noted later, indicate that some of these factors may influence the occurrence of loose smut in some wheats.

LIFE HISTORY OF THE CAUSAL ORGANISM

The causal fungus (*Ustilago tritici*) hibernates as mycelium within the wheat seed, vegetates systemically, and fruits in the young wheat heads previous to their emergence, destroying most of the tissues of the spike, except the rachis (fig. 2), and producing a black powdery mass of smut spores in their stead. The infested kernels and the plants developed from them show no external evidence of the presence of the pathogene previous to the emergence of the smutted heads. Smutted and healthy heads emerge during approximately the same period, and spores from the former are disseminated through the air while the healthy heads are in bloom. (Fig. 2.) Inoculation is intraseminal, being effected by the chance falling of one or more errant spores on the ovary or stigma which is exposed through opening of the glumes in the process of blooming. Leighty and Sando (25) found that the time required for a wheat flower to open fully in blooming averaged 3 minutes and 36 seconds, while the time from beginning of opening to complete closing averaged 26 minutes and 30 seconds. It is evident, therefore, that the exposure of the flower to possible infection is of short duration. Moreover, the infection count is small. These facts doubtless explain why, as previously noted, epiphytotics of loose smut do not occur. Within a few days all the spores are blown or washed from the diseased heads, and only the naked rachises of the spikes persist. (Fig. 3.) Following its inclusion within the glumes the spore soon germinates with the protrusion of a germ tube which eventually enters the developing kernel wherein the mycelium is developed. The mycelium finally hibernates within the matured kernel, and the life cycle is thus completed.

⁶ UNITED STATES DEPARTMENT OF AGRICULTURE. BUREAU OF PLANT INDUSTRY. Op. cit.



FIGURE 2.—A, Healthy blooming head of wheat; B, smutted head. Healthy heads are in flower and spores are blown from diseased heads during approximately the same periods. Inoculation is effected by the falling of a spore upon the stigma or the ovary during the brief period of their exposure at blooming when the flowering glumes usually open for a few minutes and the anthers are extruded.

The difficulty of controlling loose smut in wheat through seed treatment becomes readily apparent in view of the fact that the smut fungus is carried inside the seed. Surface disinfectants which control stinking smut and other surface-borne smuts have proved ineffective. Treatments which thoroughly soak the seed, like the modified hot-water treatment, are necessary to kill the intraseminal mycelium of the loose-smut fungus.

REVIEW OF PREVIOUS INVESTIGATIONS ON THE CONTROL OF LOOSE SMUT IN WHEAT

A review of the literature shows that a number of different methods have been tried for controlling loose smut in wheat, namely, (1) the selection of large kernels; (2) the use of seed more than 1 year old; (3) the removal of smutted plants from the field; (4) the use of clean seed; (5) the treatment of the seed with chemicals, hot water, hot air, and steam, alone or in some combination; and (6) the selection of resistant strains and varieties. A full review of the effectiveness of these different methods was given by Appel and Riehm (2) in 1911. Since that time further studies of the methods have been made (3, 4, 8, 17, 27, 29, 32, 34, 35).⁶ As a result of these investigations it has been found that, except for the use of varieties and strains which are resistant and otherwise desirable, treatment of the seed with hot

⁶ UNITED STATES DEPARTMENT OF AGRICULTURE. BUREAU OF PLANT INDUSTRY. Op. cit.

water is the best method of control in general farm practice. Hot water may be applied in different ways (17, 35). The modified method devised by Freeman and Johnson (10) has been generally recommended during the past 20 years. This treatment, as noted previously, is practiced only to a limited extent. It is effective in control but relatively complicated and tedious to apply in comparison with other standard cereal-seed treatments. The other methods of control enumerated above, except the use of resistant varieties, have been found either impracticable of application or ineffective in control or both.

With regard to the use of resistant varieties and strains, the literature apparently fails to show that any specific tests have been made to determine through floral inoculation the resistance or susceptibility of the more important varieties grown in the United States. Differences in susceptibility exhibited by specific classes or varieties of wheat have been observed, however, by a number of investigators. Kellerman and Swingle (22) note that loose smut is said to attack summer wheats most, winter wheats less, and hard wheats and spelt least of all. According to Melchers⁷ also, the hard wheats in Kansas have less smut than the soft wheats. Freeman and Stakman (11) observed that durum wheats seem to have comparatively little loose smut, but of the common Minnesota spring wheats the bearded varieties seem to have more than the bluestems and fifes; however, no common variety showed any marked resistance to the disease. Fromme (13) also has reported that bearded wheats show uniformly more loose smut than the beardless varieties. In one case observed by him the average amount of loose smut in 20 bearded varieties was almost three times that found in 16 beardless varieties.

In specific varieties of wheat the occurrence of loose smut or its absence has been noted by Arthur (5), Kellerman (21), Maddox (28), Coons and Spragg (9), Fromme (13, 14), Tieman (36), various contributors to the Plant



FIGURE 3.—Healthy heads of wheat at the close of the flowering period, and diseased heads from which the smut spores have been blown or washed, leaving only the naked rachis of the spikes

⁷ MELCHERS, L. E. Op. cit. (See footnote 3.)

Disease Reporter,⁸ and others. The content of loose smut in the varieties observed has been reported in various ways, and an accurate summary of the data would be difficult to present. A number of varieties have been reported resistant by some and susceptible by others. It is evident, however, that only a very few varieties have been consistently reported as showing any marked degree of resistance.

Several experiments have been recorded briefly with regard to the selection of resistant strains in susceptible varieties. Strube (33) isolated a strain of Red Schlanstedt spring wheat which was said to be both highly resistant to loose smut and superior in yield and strength of stem. Recently Fromme (14) reported the results of susceptibility tests with a number of strains of Fulcaster wheat. Some have been found to be twenty times as resistant as the parent, indicating the probability of developing a highly resistant strain of this important variety.

INFECTION STUDIES

A COMPARATIVE STUDY OF THE ANTHESIS OF RESISTANT AND SUSCEPTIBLE WHEATS

It has been suggested (13) that apparent resistance in varieties may be due simply to escape of disease by virtue of a relatively cleistogamous habit of blooming, to an anthesis of shorter duration, or to both. If this were true, then in the artificial insertion of inoculum the effectiveness of this weapon of the plants' defense, as it were, would fail to receive due consideration. Appel (1) stated that immunity from loose smut in certain types of barley is due to closed flowers, and Brefeld and Falck (6) obtained abundant infection by the introduction of spores into the artificially opened flowers of a cleistogamous variety of barley in which loose smut never had been observed. In wheat, cleistogamy does not occur, except under certain environmental conditions unfavorable for the opening of the glumes, as noted by Leighty and Sando (25).

Detailed studies on the duration and degree of glume opening in the anthesis of resistant and susceptible wheats apparently have not been made. Fromme (13) noted that in a cursory examination he failed to find any differences that appeared to be significant in the blooming of the resistant Leap and the susceptible Stoner varieties. Both were observed to open during pollination.

During the period June 1-6, 1925, the writer made a comparative study of the anthesis of 25 flowers of Dawson (C. I.⁹ 6161) and an equal number of Forward (C. I. 6691) wheat. The two varieties were grown at Ithaca, N. Y., under similar conditions, and in adjacent rows 1 foot apart. Dawson is a beardless soft winter wheat with brown glabrous glumes and white kernels. It has been consistently reported very susceptible to loose smut.⁶ The plants used in this study were grown from seed from hand-inoculated flowers and contained 27.08 per cent of smutted heads. Forward also is a beardless soft winter wheat but with white glabrous glumes and red kernels. Recent surveys indicate that it may not be so highly resistant as

⁸ UNITED STATES DEPARTMENT OF AGRICULTURE. BUREAU OF PLANT INDUSTRY. Op. cit.

⁹ UNITED STATES DEPARTMENT OF AGRICULTURE. BUREAU OF PLANT INDUSTRY. Op. cit. (See footnote 3.)

⁶Numbers preceded by C. I. are accession numbers of the Office of Cereal Crops and Diseases.

reported earlier,⁶ but there seems to be no doubt that it is far more resistant than Dawson. The Forward plants used in this experiment also were grown from seed from hand-inoculated flowers. No smutted heads appeared. Observations of the anthesis were made between 7 and 9 a. m. Both the duration and the degree of opening of the glumes were measured. The latter measurement was obtained by superimposing the points of a pair of dividers over the tips of the lemma and palea at the time of blooming when the filaments were completing their elongation and the anthers were assuming a pendent position. The divider points then were pressed into a sheet of paper, and later the distance between the two imprints was measured carefully. The distance averaged 3 mm. for both varieties. The time from the beginning of opening to complete closing was approximately 26 minutes for both varieties. Near the completion of anthesis the glumes close very slowly, and it was therefore difficult to determine the precise moment at which the process was completed. In view of these facts, it is apparent that resistance to loose smut in Forward is not a function of the mechanics of anthesis. The writer had observed also that the Leap variety, for example, which in the field consistently bears a very low percentage of loose smut,⁶ continues to do so when the flowers are inoculated artificially. (Table 2.) Moreover, if the mode of anthesis was the factor upon which resistance depended, then there should be expected no significant differences in the percentages of infected plants and heads displayed by different varieties when inoculated artificially. Reference to Table 2 shows, however, that the assembled varieties, grown and inoculated by hand under similar conditions, consistently displayed wide differences in percentages of smutted heads.

It may be noted here that the date of blooming of a variety in a particular locality apparently may enable it to escape loose smut. In the very early variety Nebraska No. 28 (C. I. 5147), for example, high percentages of smutted heads and plants were obtained in the hand-inoculation tests. (Table 2.) On the other hand, loose smut has not been observed in the fortieth-acre plots of this variety at the Arlington Experiment Farm, near Washington, D. C. It seems that this is due to the fact that blooming in this variety occurs before the air has become laden with inoculum from smutted heads in the plots of later varieties. Doubtless a very late habit of blooming also would enable a susceptible variety to escape disease under normal conditions, provided that the seed sown was clean.

EFFECT OF STAGE OF ANTHESIS ON INFECTION BY *USTILAGO TRITICI*

According to Freeman and Johnson (10), "artificial inoculations of flowers with loose smut from the time when the stamens are still green to the time when the ovary is one-third its mature size are usually successful. The optimum period for artificial inoculation is at the time when the flower is in full bloom or when the ovary is just commencing to develop after fertilization." In experiments conducted by the writer, however, inoculation succeeded best when the inoculum was inserted while the pollen was still immature. This is shown in Table 1.

⁶ UNITED STATES DEPARTMENT OF AGRICULTURE. BUREAU OF PLANT INDUSTRY. Op. cit.

TABLE 1.—Effect of stage of anthesis on the infection of Stoner^a and Red Chaff^a wheats by *Ustilago tritici*

Wheat variety	Stage of anthesis at inoculation	Year plants were grown	Number of seeds sown	Plants matured		Plants smutted		Heads		
				Number	Per cent	Number	Per cent	Total number	Number smutted	Per cent smutted
Stoner	Pollen green	1923	50	35	70.0	25	71.4	303	81	26.7
Do	Pollen being shed, or a few hours thereafter.	1923	100	40	40.0	4	10.0	299	15	5.0
Red Chaff	Pollen green	1924	200	99	49.5	76	76.8	885	514	58.1
Do	Pollen being shed, or a few hours thereafter.	1924	200	108	54.0	60	55.6	1,053	444	42.2

^a According to Clark, Martin, and Ball (7), Stoner is a synonym for Fuleaster, and Red Chaff is a synonym for Goens wheat.

In the method employed by Freeman and Johnson (10), all the flowers on a head were inoculated, the stage of anthesis being designated as the average of all the flowers on the head. In the writer's method only those flowers on each head which were in the stage designated were inoculated. All others were removed in order to delimit the designated stage of anthesis as closely as possible. To this difference in method the difference in results obtained by Freeman and Johnson (10), and by the writer may be attributed, although varietal differences and other factors also may have had some influence.

In the studies which follow, as noted previously, flowers with green pollen were not inoculated. It may be noted, however, that Hussar (C. I. 4843), Leap (C. I. 4823), Purplestraw (C. I. 1915), and several other varieties which have proved to be highly resistant to loose smut invariably have continued to remain so even when inoculated while the pollen was still green.

VARIETAL RESISTANCE AND SUSCEPTIBILITY

METHODS

The ideal method of inoculation would consist in placing one or several spores of *Ustilago tritici* on the exposed ovary or stigma of a wheat flower in full bloom. This method is hardly practicable, however, because the individual flowers on a spike are wide open for only a few minutes while the duration of blooming on a head may extend from two to seven days (23, 25). After some experimentation with different methods of artificial inoculation, a modification of one of the methods of Brefeld and Falck (6) was found most satisfactory and was adopted for all the inoculation studies reported herein except where otherwise noted. It consisted in opening the flower, when necessary, and lightly touching the stigma with the tips of forceps that had been dipped in a mass of smut spores. By this method the opening and inoculation of the flower were accomplished in one operation, and after some experience it was possible to attain considerable speed. This was a vital point to consider in view of the large number

of flowers to be inoculated during the short period in which blooming occurs. In 1923, 1924, and 1925 spores were taken from single smutted heads selected at random as needed from different plots. In 1926 and 1927 a large number of smutted heads from different plots were first collected, then the smut was taken from the heads and sifted through a fine screen. The composite sample thus obtained was used for inoculating the different varieties. The sifted smut was placed in a small capsule held in a clamp fastened to a ring. The device, ordinarily used for carrying pollen, is described by Leighty and Sando (26).

Inoculations were made on flowers which were (1) open, (2) on the verge of opening, or (3) had opened shortly before. To this end there were selected heads which contained in the lower two-thirds of the upper half of the head a relatively large number of flowers which were open or which had opened shortly before, as indicated by the unbleached extruded anther sacs and the still plumose condition of the stigma. As the flowers in this portion of the head are the first to open (25), the heads so selected therefore also contained in the adjacent upper and lower portions of the head a relatively large number of flowers on the verge of opening, as indicated by the color of the pollen sacs in the particular variety at hand. Many of these flowers on the verge of opening were stimulated to open as a result of handling the head in the process of inoculation. Freeman and Johnson (10) have shown that the period during which wheat flowers are susceptible to infection extends from a time when the pollen is still immature until the fertilized ovary has attained approximately one-third of its mature size. In the method here employed, therefore, the inoculations were made during a period which fell well within the limits of possible infection. All flowers not inoculated were removed. As a rule only the two lower flowers in a spikelet were inoculated and the upper flower or flowers were removed. At the distal portions of the heads a few entire spikelets generally were detached. Flowers were inoculated on plants grown both in the field and in the greenhouse, but most of the inoculations were made in the field. This is shown in Table 2. Each inoculated head was tagged, harvested at maturity, and threshed in a small machine especially constructed so that every kernel was recovered.

The seed was sown by hand. Individual kernels were spaced 6 inches apart in rows 1 foot apart when sown in the field, or 2 inches apart in rows 6 inches apart when sown in the greenhouse. Through this method of spacing the seed the full complement of heads of each of the individual plants always was easy to recognize. Most of the sowings, as noted in Table 2, were made in the field. The crop of 1925 was grown at Ithaca, N. Y., and previous and later crops were grown at the Arlington Experiment Farm, Rosslyn, Va. The wheats were sown in autumn during the usual sowing period at these places. All of the sowings were made with seed from the preceding crop.

In the selection of varieties for inoculation particular attention was paid to the soft red winter common wheats. Varieties in this group are most extensively grown in the middle States east of the Mississippi (24) where loose smut is most prevalent⁶ and usually are suitable for

⁶ UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. Op. cit.

growth in the environment of Rosslyn, Va., or Ithaca, N. Y., where the tests were conducted.

For some varieties listed in Table 2, the total number of plants and heads on which the data are based is relatively small because only a small percentage of the plants matured. This was due in large measure to the following facts: (1) The plants were widely spaced, and hence matured later; (2) infected seedlings proved more susceptible to winter killing (p. 333); and (3) some of the varieties were not very well adapted to the environmental conditions of one or the other of the localities where grown.

The appearance of approximately 6 to 10 per cent or more of loose-smutted heads in a variety was arbitrarily selected as a basis for considering it susceptible and discontinuing it in the test. It was not possible to establish an inflexible basis in this regard, as the inoculation of a variety could not be delayed always until the last of the diseased heads had appeared. In a few cases a variety was temporarily discontinued because it had passed the stage suitable for inoculation during a period when time was not available for inoculating all the wheats in bloom.

MATERIALS

Most of the varieties used in these studies came from the collection assembled for the study of wheat classification by members of the Office of Cereal Crops and Diseases (?). Many of the varieties were grown from seed selected from individual heads in previous seasons and thus were pure-line selections, whereas others represent merely the mass variety, as indicated in Table 2. It may be possible, therefore, that other pure lines of these varieties would show more or less loose smut. In some of the more important varieties several strains have been tested, so that the behavior of the variety, as a whole, is fairly well indicated.

RESULTS OF VARIETAL TESTS

The data obtained in the varietal resistance and susceptibility tests are presented in Table 2. The recognized varieties are listed in alphabetical order following the nomenclature used by Clark, Martin, and Ball (?). All synonymous names of varieties are listed together in alphabetical order or in the order of their Cereal Investigations (C. I.) numbers under the recognized varietal names. The varieties tested belonged to one of the three groups—common, club, or durum. The common wheats included varieties in each of four commercial classes (hard red spring, hard red winter, soft red winter, and white wheats).

TABLE 2.—*Classified list of common, club, and durum wheats grown from seed from hand-inoculated flowers, showing number of plants and heads and percentages of loose-smutted plants and heads*

[Wheats grown at Arlington Experiment Farm, Rosslyn, Va., in 1923, 1924, 1925, 1927, and 1928, and at Ithaca, N. Y., in 1925. Except when the year is followed by a footnote reference, the flowers were inoculated on plants growing in the field and the seeds which developed were sown in the field]

Variety and synonyms	C. I. No.	Pure line or mass selection	Classification	Year in which plants were grown	Total plants	Plants smutted	Total heads	Heads smutted
					Number	Per cent	Number	Per cent
Alton: Ghirka Winter.....	1438	Pure line.....	Hard red winter common.	1924	59	69.49	514	47.66
Baeska: Wisconsin Pedigree No. 408.	6156	do.....	do.....	1925			1,002	2.30
Do.....	6156	do.....	do.....	1927	55	0	145	0
Beloglina: Beloglina.....	1667	do.....	do.....	1926			856	8.76
Big Frame: Big Frame.....	6184	do.....	Soft red winter common.	1926			576	5.73
Blackhull: Blackhull.....	6251	Mass selection	Hard red winter common.	1925	11	0	124	0
Do.....	6251	do.....	do.....	1926			292	0
Do.....	6251	do.....	do.....	1927	6	0	18	0
Brown Fife: Brown Fife.....	1933	do.....	Soft red winter common.	1923	69	57.97	572	22.02
Buffum No. 17: Buffum No. 17.....	3330	Pure line.....	do.....	1926			134	8.21
China: China.....	180	Mass selection	do.....	1923	75	54.66	585	23.41
Pennsylvania Blue-stem.	4816	Pure line.....	do.....	1924	41	78.04	451	34.58
Climax: K. B. No. 2.....	4835	do.....	do.....	1926			556	6.83
Currell: Currell's Prolific.....	3326	Mass selection	do.....	1923	82	63.41	598	31.60
Golden Chaff.....	5578	Pure line.....	do.....	^a 1924	18	33.33	160	4.37
Do.....	5578	do.....	do.....	1925	25	4.00	148	1.35
Do.....	5578	do.....	do.....	1926			426	11.97
Dawson: Dawson Golden Chaff.....	—	Mass selection	White common.	1925	48	27.08	358	12.57
Do.....	3342	Pure line.....	do.....	^a 1924	85	0	669	0
Do.....	3342	do.....	do.....	1925	25	0	132	0
Do.....	3342	do.....	do.....	1925		0	57	0
Do.....	3342	do.....	do.....	^b 1926		0	49	0
Do.....	3342	do.....	do.....	1927	63	23.81	384	11.20
Do.....	3342	do.....	do.....	1928	64	95.32	651	36.10
Do.....	3342	do.....	do.....	^b 1928	97	85.57	97	85.57
Honor.....	6161	Mass selection	do.....	1923	67	44.77	525	16.00
Democrat: Democrat.....	3384	Pure line.....	do.....	1924	63	47.61	731	21.06
Diamond Grit: Diamond Grit.....	3385	do.....	Soft red winter common.	1924	23	47.82	231	23.27
Diehl-Mediterranean: Diehl-Mediterranean.....	1395	do.....	do.....	1925			521	5.76
Do.....	1395	do.....	do.....	1927	61	72.13	130	50.77
Eaton: Eaton.....	4682	do.....	White common.	1925	2	50.00	5	20.00
Eureka: Eureka.....	5170	Mass selection	Hard red winter common.	1925	112	66.07	908	30.18
Evans: Evans.....	2946	do.....	Soft red winter common.	1923	64	0	387	0
Do.....	2946	do.....	do.....	1924	50	20.00	345	16.52
Flint: Flint.....	6612	do.....	do.....	1924	97	46.39	522	33.90

^a The flowers were inoculated on plants growing in the greenhouse, and the seed which developed was sown in the field.

^b The flowers were inoculated on plants growing in the field, and the seed which developed was sown in the greenhouse.

TABLE 2.—Classified list of common, club, and durum wheats grown from seed from hand-inoculated flowers, showing number of plants and heads and percentages of loose-smutted plants and heads—Continued

Variety and synonyms	C. I. No.	Pure line or mass selection	Classification	Year in which plants were grown	Total plants	Plants smutted	Total heads	Heads smutted
					Number	Per cent	Number	Per cent
Forward:								
Forward	6691	Mass selection	Soft red winter common.	1923	61	0	351	0
Do	6691	do	do	1924	22	0	106	0
Do	6691	do	do	1925	64	0	489	0
Fulcaster:								
Bearded Purple-straw	1911	do	do	1923	37	0	218	0
Do	1911	do	do	1924	57	26.31	369	6.23
Dietz Longberry	1981	do	do	1923	40	2.50	306	1.96
Do	1981	do	do	1924	37	43.24	193	15.54
Fulcaster	—	do	do	1925	50	8.00	362	1.10
Do	6162	do	do	1923	37	18.91	212	23.58
Marvelous	3605	do	do	1923	49	0	272	0
Do	3605	do	do	1924	46	2.17	269	1.11
Do	3605	do	do	1925	26	0	117	0
Stoner	2980	do	do	1923	75	38.66	602	15.94
Fultz:								
Fultz	1923	do	do	1923	54	37.03	412	15.53
Do	3416	Pure line	do	1924	82	0	893	0
Do	3416	do	do	1925	54	0	388	0
Do	3416	do	do	1926	—	—	458	1.53
Do	3416	do	do	1927	202	37.13	842	18.29
Fultz-Mediterranean:								
Fultz-Mediterranean	5813	Mass selection	do	1923	87	25.28	621	14.33
Genesee Giant:								
Early Genesee Giant	1744	Pure line	White common	1925	56	35.71	206	17.96
Gipsy:								
Defiance	5305	do	Soft red winter common.	1925	78	0	560	0
Gipsy	—	do	do	1925	44	0	516	0
Do	—	do	do	1926	—	—	528	0
Do	—	do	do	1927	257	6.23	831	2.17
Do	—	do	do	1928	39	61.54	392	17.85
Do	3439	Mass selection	do	1923	87	1.14	611	.15
Do	3439	do	do	1924	80	30.00	563	10.47
Gladden:								
Gladden	5577	do	do	1923	79	7.59	715	4.47
Do	5577	do	do	1924	70	20.00	423	8.51
Goens:								
Goens	3428	do	do	1923	6	100.00	23	95.65
Red Chaff	—	do	do	1924	207	57.00	1,940	49.38
Goldcoin:								
Goldcoin	—	do	White common	1925	98	23.47	671	6.86
Junior No. 6	6971	do	do	1925	139	26.62	1,014	8.38
Golden Cross:								
Golden Cross	—	do	Soft red winter common.	1925	80	37.50	536	8.96
Grandprize:								
St. Louis Grand-prize	4876	Pure line	do	1924	44	47.72	258	33.72
Harvest Queen:								
Harvest Queen	—	do	do	1925	35	77.15	404	66.84
Do	—	do	do	1928	34	76.47	34	76.47
Do	5314	do	do	1925	56	0	405	0
Red Cross	4882	Mass selection	do	1923	53	0	332	0
Do	4882	do	do	1924	90	1.11	465	.21
Do	4882	do	do	1925	59	11.86	418	7.89
Do	4882	Pure line	do	1924	115	.86	1,092	.09
Do	4882	do	do	1925	98	1.02	809	1.24
Haynes Bluestem:								
Haynes Bluestem	2874	Mass selection	Hard red spring common.	1923	71	33.80	515	20.38
Humpback:								
Humpback	3690	Pure line	do	1924	30	30.00	186	16.66

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^b The flowers were inoculated on plants growing in the field, and the seed which developed was sown in the greenhouse.

TABLE 2.—*Classified list of common, club, and durum wheats grown from seed from hand-inoculated flowers, showing number of plants and heads and percentages of loose-smutted plants and heads—Continued*

Variety and synonyms	C. I. No.	Pure line or mass selection	Classification	Year in which plants were grown	Total plants	Plants smutted	Total heads	Heads smutted
					Number	Per cent	Number	Per cent
Hussar:					179	0	662	0
Red Hussar.....	4843	Pure line.....	Hard red winter common.	• 1924				
Do.....	4843	do.....	do.....	• 1925	220	.45	1,102	.64
Hybrid 128:								
Hybrid 128.....	4229	do.....	Club.....	• 1924	163	93.25	528	89.58
Illini Chief:								
Illini Chief.....	5406	do.....	Soft red winter common.	1923	69	27.53	513	16.76
Imperial Amber:								
Imperial Amber.....	5338	do.....	do.....	1924	68	63.23	492	40.04
Jenkin:								
Jenkin Club.....	5177	do.....	Club.....	• 1924	138	97.10	463	97.62
Jones Fife:								
Jones Fife.....	4468	do.....	Soft red winter common.	• 1924	134	44.77	399	30.32
Kanred:								
Kanred.....	5146	do.....	Hard red winter common.	1925	62	3.23	464	.65
Do.....	5146	do.....	do.....	1926			535	5.23
Do.....	5146	do.....	do.....	1927	65	0	166	0
Kinney:								
Kinney.....	5189	do.....	Hard red spring common.	• 1924	24	75.00	279	68.10
Kota:								
Kota.....	5878	do.....	do.....	• 1924	90	95.55	487	93.42
Leap:								
Leap's Prolific.....	4823	do.....	Soft red winter common.	• 1924	112	0	926	0
Do.....	4823	do.....	do.....	1925	62	6.45	352	1.42
Do.....	4823	do.....	do.....	1928	62	9.67	595	2.69
Link:								
Missing Link.....	4866	do.....	White common.	1926			314	12.74
Little Club:								
Little Club.....	4066	do.....	Club.....	• 1924	160	97.50	651	97.08
Lofthouse:								
Lofthouse.....	3275	do.....	Soft red winter common.	1926			60	3.33
Do.....	3275	do.....	do.....	1927	151	17.88	727	5.50
Longberry No. 1:								
Jones Longberry.....	3451	Mass selection.	White common.	1925	57	40.35	227	25.55
Mammoth Amber:								
Mammoth Amber.....	3355	Pure line.....	do.....	1926			467	16.70
Mammoth Red:								
Mammoth Red.....	2008	Mass selection.	Soft red winter common.	1923	29	0	182	0
Do.....	2008	do.....	do.....	1924	66	18.18	390	7.43
Marquis:								
Marquis.....	3641	Pure line.....	Hard red spring common.	• 1924	149	23.48	458	17.68
Martin:								
Martin.....	4463	do.....	White common.	• 1924	105	68.57	292	67.80
Mealy:								
Mealy.....	3358	do.....	Soft red winter common.	1924	67	4.47	661	.81
Do.....	3563	Mass selection.	do.....	1923	38	0	267	0
Do.....	3563	do.....	do.....	1924	91	21.97	594	11.61
Mediterranean:								
Missouri Bluestem.....	1912	do.....	do.....	1923	71	0	622	0
Do.....	1912	do.....	do.....	1924	78	29.48	681	11.89
Rocky Mountain.....	1930	do.....	do.....	1923	65	24.61	759	7.24
Minhardi:								
Minhardi.....	5149	Pure line.....	do.....	1926			578	18.17
Minturki:								
Minturki.....	6155	do.....	Hard red winter common.	1926			928	6.90

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* The flowers were inoculated on plants growing in the greenhouse, and the seed which developed was sown in the greenhouse.

TABLE 2.—Classified list of common, club, and durum wheats grown from seed from hand-inoculated flowers, showing number of plants and heads and percentages of loose-smutted plants and heads—Continued

Variety and synonyms	C. I. No.	Pure line or mass selection	Classification	Year in which plants were grown	Total plants	Plants smutted	Total heads	Heads smutted
Nebraska No. 28: Nebraska Hybrid	5147	Mass selection	Soft red winter common.	1923	Number 73	Per cent 57.53	Number 543	Per cent 45.48
Nigger:								
Nigger	5689	do.	do.	1923	82	41.46	855	21.52
Nittany (Pa. No. 44):								
Nittany	6882	Mass selection	do.	1923	72	0	398	0
Do.	6882	do.	do.	1924	34	8.82	227	6.60
Do.	6882	do.	do.	1925	51	0	259	0
Do.	6882	Pure line	do.	1925	64	18.75	538	8.18
O. A. C.:								
O. A. C. No. 104	6983	do.	White common.	1925	64	87.50	476	52.52
Oatka Chief:								
Oatka Chief	3481	Pure line	do.	1926			480	14.17
Odessa:								
Odessa	6027	do.	Soft red winter common.	" 1924	29	31.03	289	19.03
Do.	6027	do.	do.	1924	81	9.87	800	8.00
Ontario Wonder:								
Ontario Wonder	3483	do.	do.	1926			356	19.10
Penquite:								
Velvet Chaff	5948	do.	do.	1924	98	0	681	0
Do.	5948	do.	do.	1925	51	0	320	0
Do.	5948	do.	do.	1926			235	0
Do.	5948	do.	do.	1927	353	80.74	2,541	39.16
Do.	5948	do.	do.	1928	71	88.74	744	37.91
Do.	5948	do.	do.	" 1928	95	84.21	152	86.18
Pentad:								
Pentad (D-5)	3322	do.	Durum	" 1924	8	0	6	0
Do.	3322	do.	do.	" 1925	98	1.02	225	.41
Pesterboden:								
Weissenburg	1563	do.	Hard red winter common.	1924	57	68.42	706	28.18
Peterson:								
Lars Peterson	5538	do.	Soft red winter common.	" 1924	27	70.37	196	25.00
Poole:								
Poole	3488	do.	do.	" 1924	106	.94	1,227	.08
Do.	3488	do.	do.	1924	31	3.22	215	1.86
Do.	3488	do.	do.	1925	54	0	239	0
Do.	3488	do.	do.	1926			398	6.78
Power:								
Power's Fife	3697	Mass selection	Hard red spring common.	1923	19	31.57	105	24.76
Do.	3697	Pure line	do.	" 1924	63	61.90	600	44.33
Prelude:								
Wisconsin Wonder	4323	Mass selection	do.	" 1925	286	46.85	1,032	31.88
Preston:								
Preston	3328	Pure line	do.	1924	72	4.16	715	1.53
Velvet Chaff	3081	do.	do.	" 1924	90	3.33	311	1.60
Do.	3081	do.	do.	" 1925	140	.71	555	.36
Prohibition:								
Prohibition	4068	do.	White common.	1925	22	22.73	103	11.65
Prosperity:								
American Bronze	5380	do.	Soft red winter common.	" 1924	48	52.08	335	16.41
Purplestraw:								
Purplestraw	1915	Mass selection	do.	1923	27	0	176	0
Do.	1915	do.	do.	1924	56	5.35	426	1.40
Do.	1915	do.	do.	1925	54	3.70	265	.75
Do.	1957	do.	do.	1923	19	0	119	0
Do.	1957	do.	do.	1924	42	2.38	302	.99
Do.	1957	do.	do.	1925	39	28.21	218	11.47
Red Chief:								
Early Red Chief	3392	Pure Line	do.	1924	32	21.87	233	7.29

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TABLE 2.—*Classified list of common, club, and durum wheats grown from seed from hand-inoculated flowers, showing number of plants and heads and percentages of loose-smutted plants and heads—Continued*

Variety and synonyms	C. I. No.	Pure line or mass selection	Classification	Year in which plants were grown	Total plants	Plants smutted	Total heads	Heads smutted
					<i>Number</i>	<i>Per cent</i>	<i>Number</i>	<i>Per cent</i>
Red Clawson:					69	15.94	552	6.34
Early Red Clawson.	5349	Mass selection.	Soft red winter common	1923				
Do.	5349	do.	do.	1924	96	12.50	466	4.07
Red May:								
Beechwood.	4886	Pure line.	do.	^a 1924	33	39.39	375	14.40
Early Ripe.	5319	Mass selection.	do.	1923	79	55.69	620	22.09
Jones Longberry.	5339	Pure line.	do.	1925	39	74.36	287	40.42
Michigan Amber.	4864	Mass selection.	do.	1923	70	64.28	486	22.22
Michigan Wonder.	—	Pure line.	do.	1928	42	59.53	520	23.85
Do.	—	do.	do.	^b 1928	58	55.17	83	42.17
Red May.	5336	Mass selection.	do.	1923	75	14.66	505	4.75
Do.	5336	do.	do.	1924	21	9.52	123	3.25
Do.	5336	do.	do.	1925	27	33.33	168	6.55
Red Rock:								
Red Rock.	5976	do.	do.	1923	23	73.91	206	23.78
Do.	5976	Pure line.	do.	1925	84	66.67	722	24.10
Red Russian:								
Red Russian.	4509	do.	do.	^a 1924	8	0	44	0
Do.	4509	do.	do.	1925	26	11.54	147	5.44
Do.	4509	do.	do.	1926	—	—	48	10.42
Red Wave:								
Red Wave.	—	Mass selection.	do.	1925	102	74.51	626	37.06
Do.	5804	do.	do.	1923	35	45.71	145	24.13
Rice:								
Rice.	5734	Pure line.	do.	^a 1924	39	5.12	287	3.83
Do.	5734	do.	do.	1924	71	9.85	523	7.45
Ridit:								
Smutproof.	6703	do.	Hard red winter common.	^c 1924	118	0	496	0
Do.	6703	do.	do.	^c 1925	183	0	947	0
Rudy:								
Rudy.	5656	Mass selection.	Soft red winter common.	1923	73	53.42	668	14.97
Rupert:								
Rupert's Giant.	5920	Pure line.	do.	^a 1924	106	66.03	778	21.20
Rural New Yorker								
No. 6:	3496	do.	do.	^a 1924	33	39.39	267	11.23
Rural New Yorker								
No. 57:	3516	do.	do.	1926	—	—	850	27.17
Russian:								
Russian.	5737	do.	do.	1924	71	23.94	617	4.37
Do.	5737	do.	do.	1925	80	16.25	617	4.54
Do.	5737	do.	do.	1926	—	—	1,020	1.47
Do.	5737	do.	do.	1927	270	7.77	1,415	2.69
Seneca Chief:								
Seneca Chief.	3575	do.	White common.	1924	39	69.23	392	15.81
Shepherd:								
Shepherd.	6163	do.	Soft red winter common.	1924	20	5.00	196	.51
Do.	6163	do.	do.	1925	30	0	225	0
Do.	6163	do.	do.	1926	—	—	377	0
Do.	6163	do.	do.	1927	300	42.33	1,968	14.99
Sibley:								
Sibley New Golden.	5666	do.	do.	1924	40	5.00	445	1.57
Do.	5666	do.	do.	1925	34	5.88	255	2.35
Do.	5666	do.	do.	1926	—	—	295	3.39
Do.	5666	do.	do.	1927	152	21.05	717	10.74
Silvercoin:								
Silvercoin.	6013	do.	White common.	1924	44	61.36	304	17.76
Silversheaf:								
Jones Silver Sheaf	2496	do.	Soft red winter common.	1924	32	3.12	259	.38
Longberry.	2496	do.	do.	1925	37	0	212	0
Do.	2496	do.	do.	1926	—	—	94	0
Do.	2496	do.	do.	1927	333	47.15	1,990	18.49

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					Number	Per cent	Number	Per cent
Sol:								
Sol.....	6009	Pure line.....	Soft red winter common.	1926			663	0
Do.....	6009	do.....	do.....	1927	277	0	735	0
Treadwell:								
Treadwell.....	5332	do.....	White common.	1926			733	29.74
Trumbull:								
Trumbull.....	5657	do.....	Soft red winter common.	1924	34	0	448	0
Do.....	5657	do.....	do.....	1925	1	0	1	0
Turkey:								
Red Russian.....	3497	Mass selection.	Hard red winter common.	1923	68	83.82	477	44.23
Theiss.....	1561	do.....	do.....	1923	52	0	212	0
Do.....	1561	do.....	do.....	1924	80	31.25	352	15.05
Turkey.....	1558	Pure line.....	do.....	1924	174	54.02	775	46.45
Do.....	6152	Mass selection.	do.....	1923	72	47.22	824	7.03
Wheedling:								
Wheedling.....	4846	Pure line.....	Soft red winter common.	1924	64	31.25	633	6.31
White Fife:								
White Fife.....	4412	do.....	White common.	1924	54	55.55	341	17.88
White Odessa:								
White Odessa.....	4651	do.....	do.....	1924	131	29.00	353	16.99
White Winter:								
White Winter.....	5219	do.....	do.....	1925	29	3.45	160	1.88
Do.....	5219	do.....	do.....	1926			583	.01
Do.....	5219	do.....	do.....	1927	60	30.00	109	11.93
Windsor:								
Extra Early Windsor.....	5915	do.....	do.....	1925	73	52.05	389	24.16
Winter Bluestem:								
Winter Bluestem.....	5409	do.....	do.....	1926			84	21.43
Wyandotte:								
Wyandotte Red.....	3549	do.....	Soft red winter common.	1924	19	0	176	0
Do.....	3549	do.....	do.....	1925	14	14.29	83	4.82
Zimmerman:								
Zimmerman.....	2907	do.....	do.....	1924	45	31.11	254	26.77

^a The flowers were inoculated on plants growing in the greenhouse, and the seed which developed was sown in the field.

^b The flowers were inoculated on plants growing in the field, and the seed which developed was sown in the greenhouse.

^c The flowers were inoculated on plants growing in the greenhouse, and the seed which developed was sown in the greenhouse.

Table 2 shows that Pentad (C. I. 3322), the only durum wheat tested, was highly resistant to loose smut. The three club wheats included in the test, Hybrid 128 (C. I. 4229), Jenkin (C. I. 5177), and Little Club (C. I. 4066), were strikingly susceptible, the latter two producing over 97 per cent of smutted plants and heads. (Fig. 4.) This is noteworthy in view of the fact that these wheats produce little or no loose smut in the Pacific Coast States, where they are grown extensively. It is probable that the extreme dryness of the atmosphere at infection time, i. e., when the wheats are in bloom, plays an important rôle in this connection. That higher humidities are favorable for infection is indicated by the fact that loose smut in these States is found in irrigated sections and also by the unusually high content of loose smut in the plants grown from seed from flowers inoculated under the humid conditions of the greenhouse. According to Tisdale et al. (37), the selections of Hybrid 128, Jenkin, and Little Club, noted above, as well as other selections, proved very susceptible to bunt also.



FIGURE 4.—Hybrid 128 (C. I. 4229), a club wheat grown from infected seed in a greenhouse at Arlington Experiment Farm, Rosslyn, Va. It produced 93.25 per cent of smutted plants, 89.58 per cent of smutted heads and many badly smutted leaves. The other club wheats included in the varietal test, Jenkin (C. I. 5177), and Little Club (C. I. 4066), produced over 97 per cent of smutted plants and heads. Many of the leaves also were badly smutted and distorted

With regard to the common wheats, Table 2 shows that the range of resistance ran from almost complete susceptibility to immunity. Resistant and susceptible varieties were found within each of the four commercial groups, except that no resistant variety was found in the white wheats included in the test.

The varieties tested in each of the groups, which produced 5 per cent or less of smutted heads in each of the two or three years when grown, are listed in Table 3, and grouped in their respective commercial classes. Not included in the list are five varieties which produced less than 5 per cent smutted heads, but which have been tested in one year only. These varieties are: Fulcaster (C. I. —), Gipsy (C. I. 5305) Harvest Queen (C. I. 5314), and Mealy (C. I. 3358), soft red winter wheats; and Preston (C. I. 3328), a hard red spring wheat.

TABLE 3.—*Classified list of common wheats which produced 5 per cent or less of smutted heads in each of the years when grown from seed from hand-inoculated flowers at Rosslyn, Va., or Ithaca, N. Y.*

HARD RED SPRING WHEATS

Variety and synonym	C. I. No.	Pure line or mass selection	Number of years tested	Total number of heads	Average percentage of smutted heads
Preston: Velvet Chaff.....	3081	Pure line.....	2	866	0.81

HARD RED WINTER WHEATS

Bacskai: Wisconsin Pedigree No. 408.....	6156	Pure line.....	2	1,147	2.01
Blackhull: Blackhull.....	6251	Mass selection.....	3	434	0
Hussar: Red Hussar.....	4843	Pure line.....	2	1,764	.40
Ridit: Smutproof.....	6703	do.....	2	1,443	0

SOFT RED WINTER WHEATS

Forward: Forward.....	6691	Mass selection.....	3	946	0
Fulcaster: Marvelous.....	3605	do.....	3	658	.46
Leap: Leap's Prolific.....	4823	Pure line.....	3	1,873	1.12
Purplestraw: Purplestraw.....	1915	Mass selection.....	3	867	.92
Russian: Russian.....	5737	Pure line.....	3	3,669	2.94
Sol: Sol.....	6009	do.....	2	1,398	0
Trumbull: Trumbull.....	5657	do.....	2	449	0
Wyandotte: Wyandotte Red.....	3549	do.....	2	259	1.54

The occurrence of loose smut in many of the important wheats has been frequently reported in the Plant Disease Bulletins.⁶ Examination of these reports shows that the varieties Blackhull, Forward, Leap, and Trumbull consistently have proved resistant in the States where grown. Reference to Table 2 shows that these varieties also

were resistant when artificially inoculated. Likewise a number of varieties which have been uniformly reported highly susceptible also have proved highly susceptible in the varietal tests. Examples are: Currell, Dawson, Fulcaster (Stoner), Goens (Red Chaff), Kota, Red May (Michigan Amber), Red Wave, and Red Rock. (Tables 2 and 3.) Further examination of the data in the Plant Disease Bulletins⁶ shows that certain varieties have been reported resistant in some localities and susceptible in others. Fulcaster, Fultz, Kanred, and Prelude (Wisconsin Wonder) are examples. These different findings may be due wholly or in part to a mixture of varieties, mistakes in identification of varieties, effects of different interactions of environmental factors in the various localities where the varieties were observed, particular strains of the causal organism with different powers of infection, particular strains of the wheat with different susceptibilities, or other causes. With regard to varieties with resistant and susceptible strains, reference to Table 2 shows that in Fulcaster, Gipsy, Harvest Queen, Mealy, Purplestraw, and Red May, some strains thus far have proved resistant while others are susceptible. Fromme (14) also has called attention to the existence in Fulcaster of races with different degrees of susceptibility. These findings lend encouragement to the possibility of reducing loose smut in the important susceptible wheats through the use of resistant pure-line selections.

The resistance of certain varieties in some localities and their susceptibility in others also may be due to the existence of physiologic forms of the causal fungus. Very recently Piekenbrock (30) has presented evidence to show that physiologic forms exist in the loose-smut fungus of wheat. In the course of the varietal tests the writer also has obtained evidence indicating their presence. For example, the varieties Fultz (C. I. 3416), Shepherd (C. I. 6163), Sibley (C. I. 5666), Silversheaf (C. I. 2496), and White Winter (C. I. 5219) were highly resistant or immune in 1926 and previously, but in 1927 they proved highly susceptible. Dawson (C. I. 3342), and Penquite (C. I. 5948) were immune in 1924, 1925, and 1926, but in 1927 and 1928 these pure-line selections proved highly susceptible. It is interesting to note, however, that Russian (C. I. 5737), highly resistant in 1924, 1925, and 1926, produced only 2.69 per cent of smutted heads in 1927. Flowers of Russian were inoculated in 1926 with the same composite smut sample used in the inoculation of the other wheats. The explanation of the sudden change in the reaction of the mentioned varieties in 1927 may be due to the fact that in 1923, 1924, and 1925 inoculum was taken from single heads, while in 1926 and 1927, as noted previously (p. 321), the inoculum was a composite sample of sifted smut from many different varieties. It seems probable, therefore, that one or more new and virulent forms may have been introduced in the sample, or a potent hybrid form may have been produced as a result of fusion of mycelia from different forms following inoculation.

The pure-line selections of Hussar (C. I. 4843) and Ridit (C. I. 6703) (Tables 2 and 3) used in the varietal test proved, respectively, highly resistant to and immune from loose smut. In experiments on the resistance of wheat varieties to stinking smut in the Pacific Coast States reported by Tisdale et al. (37), these same selections were used.

⁶ UNITED STATES DEPARTMENT OF AGRICULTURE. BUREAU OF PLANT INDUSTRY. Op. cit.

Hussar was immune from stinking smut, and Ridit was highly resistant. These wheats, therefore, may prove valuable in breeding in sections of the country where both loose smut and stinking smut cause appreciable damage.

The results of the studies of resistance and susceptibility in the varieties shown in Table 2 may be summarized as follows: (1) Pentad, the only durum wheat tested, was highly resistant to loose smut; (2) the three club wheats tested (Hybrid 128, Jenkin, and Little Club) were highly susceptible, which is noteworthy in view of the fact that they produce little or no loose smut in the Pacific Coast States where they are grown extensively; (3) in the common wheats, the range of resistance extends from high susceptibility to apparent immunity; the highly resistant and immune varieties are relatively few, but one or more were found in each of the four commercial groups except the white wheats; (4) both resistant and susceptible strains were found in the important varieties Fulcaster, Gipsy, Harvest Queen, and Red May.

ACIDITY AND VARIETAL RESISTANCE OF WHEAT TO LOOSE SMUT

In recent years there has been considerable speculation regarding a possible interrelationship between resistance to disease and the concentration of hydrogen ions and titratable acid in the cell sap of the plant. Several varieties listed in Table 2 were grown from the same lots of seed used by Hurd in studies of acidity and varietal resistance of wheat to bunt, stem rust, and other diseases (18, 19, 20). The data, therefore, may be examined for possible correlations between resistance or susceptibility to loose smut and the corresponding acidity determinations reported by Hurd.

In studies of acidity with reference to stem rust, Hurd (18) found that the varieties Kota (C. I. 5878), Little Club (C. I. 4066), Pentad (C. I. 3322), and Preston (C. I. 3081) showed no evidence of significant differences in either hydrogen-ion or titratable-acid concentration at any stage of their development. In fact, the values were strikingly similar for varieties so unlike both in morphological type and susceptibility to stem rust (in general, Kota and Pentad are resistant and Little Club and Preston are susceptible). The resistance or relative susceptibility of each of these wheats to loose smut is shown in Table 4. As noted previously (Table 2), the varieties were pure-line selections. The plants were grown in the greenhouse from seed from hand-inoculated flowers.

TABLE 4.—The resistance or susceptibility to loose smut shown by four wheats having cell sap with similar hydrogen-ion and titratable-acid concentrations

Variety	C. I. No.	Classification	Year grown	Total number of plants	Number of plants smutted	Percentage of plants smutted	Heads		
							Total number	Number smutted	Percent smutted
Kota.....	5878	Hard red spring common.	1924	90	86	95.55	487	455	93.42
Little Club...	4066	Club.....	1924	100	156	97.50	651	632	97.08
Pentad.....	3322	Durum.....	{ 1924 1925 }	103	1	.94	271	1	.37
Preston.....	3081	{Hard red spring common.}	{ 1924 1925 }	230	4	1.74	866	7	.81

Pentad and Preston are very resistant. Kota and Little Club are highly susceptible. It is evident, therefore, that no relation exists between the acid values of these varieties and their resistance or susceptibility to loose smut.

In studies of acidity of 1-week-old wheat seedlings and varieties variously resistant to bunt, Hurd-Karrer (20) again found no relation between either the hydrogen ion or the titratable acidity and resistance. She found that Hussar (C. I. 4843), Little Club (C. I. 4066), Ridit (C. I. 6703), and White Odessa (C. I. 4651) were characterized by a relatively high titratable acidity. The data in Table 2 show that Ridit is apparently immune to loose smut, Hussar is highly resistant, White Odessa is moderately resistant, and Little Club is extremely susceptible. Hurd-Karrer (20) also found that the varieties Hybrid 128 (C. I. 4229), Jenkin (C. I. 5177), and Jones Fife (C. I. 4468) were characterized by a relatively high hydrogen-ion concentration. Table 2 shows that Jones Fife is moderately susceptible and Hybrid 128 and Jenkin are highly susceptible to loose smut. The susceptibility of these varieties, and of Little Club with its relatively high titratable acidity, is further evidence that there is no causal relation between high acid concentration in the juice and ability to resist the invasion of *Ustilago tritici*.

PHYSIOLOGIC STUDIES

The foregoing results have shown that inherent differences in wheat varieties alone may be held accountable for differences in the percentage of loose-smutted plants and heads, and that these differences may range from 0 to almost 100 per cent.

That some environmental factors may have an important influence on the percentage of smuttedness within a variety also seems evident from the work of a number of investigators, as noted later, and from the experience of seed growers. With regard to the latter, the writer's attention frequently has been called to cases in which the variously distributed portions of a single lot of seed, sown at different times and on different soils, produced widely varying percentages of smutted heads. It has been the object of these studies, therefore, to ascertain the *modus operandi* of some of the factors and the possibility of decreasing loose smut in wheat through changes in agronomic practice which might reduce or completely control the disease.

INFLUENCE OF WINTERKILLING ON THE OCCURRENCE OF LOOSE SMUT

It has been noted by Coons and Spragg (9) in Michigan that infected wheat seems more likely to succumb to winterkilling than healthy wheat. In Virginia, Fromme¹⁰ also has noted that infected seedlings seem less able to survive a severe winter. The relation between infection and susceptibility to winterkilling was studied by the writer in an experiment begun at the Arlington Experiment Farm, Rosslyn, Va., where the winters are relatively mild, and completed at Ithaca, N. Y., where the winters are relatively severe.

In the Virginia test, conducted in 1920-21, 72 different wheat lots representing 45 recognized varieties were used. Equal numbers of healthy and infected seed of each lot were sown in alternate rows. The number of seedlings which emerged before winter and the number

¹⁰ FROMME, F. D. Op. cit. (See footnote 3.)

of plants which survived the winter and matured were recorded, as well as the percentage of smutted plants appearing in the latter. In the New York tests conducted in 1922-23 and 1924-25 a similar procedure was followed, but only a few varieties were used. The results in summarized form are presented in Table 5.

TABLE 5.—*Relative susceptibility of wheat seedlings from healthy and smut-infected seed to winterkilling when grown at Rosslyn, Va., and Ithaca, N. Y.*

Year	Location of experiment	Number of—		Condition of seed	Seeds sown	Germination	Seedlings before winter	Plants matured	Plants winterkilled		Smutted plants	Smutted heads
		Varieties	Lots						Number	Per cent		
1920-21	{ Rosslyn, Va. }	45	72	(Uninfected.....)	1,857	76.36	1,418	1,300	118	8.32	0	0
				(Infected.....)	1,857	79.43	1,475	1,299	176	11.93	24.63	16.66
1922-23	{ Ithaca, N. Y. }	3	3	(Uninfected.....)	230	89.57	206	147	59	28.64	0	0
				(Infected.....)	230	86.52	199	81	118	59.30	25.93	22.52
1924-25	do	2	2	(Uninfected.....)	800	57.13	457	344	113	24.73	0	0
				(Infected.....)	800	45.88	367	245	122	33.24	31.53	30.03

In each of the three years higher percentages of winterkilling occurred in plants grown from infected seed. In 1924-25 there was less winter injury to wheat in New York than usual, and the plants were grown in a sheltered area. For these reasons the 1922-23 results doubtless are more representative of the usual condition in New York.

At Ithaca, N. Y., fewer infected than uninfected seeds germinated, as shown in Table 5. Coons and Spragg (9) also obtained a poorer stand as well as more winterkilling of the susceptible "Goings" when grown from infected seed. This was not true when the resistant Shepherd's Perfection was so grown. It seems probable, therefore, that the natural checks on loose smut in susceptible varieties, particularly in districts having severe winters, are (1) a reduction in the number of seedlings which emerge from infected seed and (2) a higher percentage of winterkilling in the emerged seedlings from such seeds.

INFLUENCE OF VEGETATIVE VIGOR OF WHEAT IN RELATION TO RESISTANCE OR SUSCEPTIBILITY TO LOOSE SMUT

The work of Raines (31) and his review of many previous investigations show that increase in vegetative vigor of susceptible plants may be accompanied by increase or decrease in virulence of the disease, depending upon the particular plant and disease in question.

In a study of the effect of fertilizers on the susceptibility of barley to loose smut, Lind (27) found that the addition of Chile saltpeter, superphosphate, and potash, alone or in various combinations, increased the percentage of loose smut on six plots out of seven. In a series of plots sown to wheat continuously and receiving different quantities of fertilizers, Fromme (12) found more loose smut in plants grown on soils of low fertility than on soils of high fertility. Similar observations were made in connection with stinking smut in greenhouse plots. Heuser (15) and Hiltner and Lang (16) obtained similar results with bunt in field-grown plants, but the latter report that certain nitrogenous fertilizers appreciably increased both the vigor of

wheat plants and the percentages of loose smut which they produced. Tieman (36) found that fertilizers had very little influence on loose smut in wheat, except that phosphoric acid when used alone increased smuttedness.

The influence of the vegetative vigor of wheat on its resistance or susceptibility to loose smut was studied further by the writer in two experiments. The first was conducted in the greenhouse in 1922-23 and the second in the field the following year. In the greenhouse experiment, infected seed of Leap, a highly resistant wheat, and Goens (Red Chaff), a very susceptible wheat, was used. One hundred and twenty kernels of each variety were sown in a very rich soil, and a like number were sown in a very poor soil. The plants in the rich soil grew very vigorously, attained an average height of 35 inches, and produced an average of 4.87 heads per plant. The heads were large and well filled. On the other hand, plants on the poor soil grew very slowly, reached an average height of only 7 inches, and produced an average of only 1.11 heads per plant. The heads were small and poorly filled. It is evident, therefore, that the plants on the rich and the poor soils were extremely different in vegetative vigor. At maturity the height of the plants and the number of healthy and smutted plants and heads were recorded. The results are presented in Table 6.

TABLE 6.—*Influence of vegetative vigor on the occurrence of loose smut in Leap and Goens (Red Chaff) wheats grown in the greenhouse*

Variety	Kind of growth	Average height of plants (inches)	Average number heads per plant	Number of seeds sown	Number of plants matured	Percentage of plants matured	Number of plants smutted	Percentage of plants smutted	Total number of heads	Number of heads smutted	Percentage of heads smutted
Leap	Vigorous	35	5.29	120	86	71.67	8	9.30	455	23	5.05
	Weak	7	1.11	120	97	80.83	3	3.09	108	3	2.78
Goens	Vigorous	35	4.55	120	114	95.00	76	66.67	519	326	62.81
	Weak	7	1.12	120	113	94.17	72	63.72	126	78	61.90
Leap and Goens	Vigorous	35	4.87	240	200	83.33	84	42.00	974	349	35.83
	Weak	7	1.11	240	210	87.50	75	35.71	234	81	34.62

When growth of the plants was vigorous, higher percentages of smutted plants and heads were produced, especially by the Leap variety. However, the average percentage of smutted heads produced by vigorous plants of both wheats (35.83) was only slightly greater than that produced by weak plants of the two varieties (34.62).

The second experiment was conducted in 1923-24. The infected seed used in the resistance studies of that year was sown in the field in four successive plots numbered 1, 2, 3, and 4. Plots 1 and 4 were adapted for use in this special study. The procedure throughout the experiment was identical except that plot 4 received an application of manure at the rate of 20 loads per acre, and of phosphate at the rate of 200 pounds per acre, several weeks before the seed was sown. On each plot, 2,251 inoculated kernels, representing 70 different lots and 60 different varieties, were sown on October 26, 1923. In the following summer a record was made of the number of healthy and smutted heads on each of the plants which matured. A summary of the results is presented in Table 7.

TABLE 7.—*Influence of vegetative vigor on severity of loose smut in 70 different lots of wheat (60 varieties) when grown in the field on fertilized and unfertilized plots*

Item	Fertilized plot		Unfertilized plot	
	Number	Per cent	Number	Per cent
Seeds sown.....	2,251		2,251	
Plants matured.....	1,062	47.17	1,176	52.24
Heads.....	10,847		9,184	
Average heads per plant.....	10.21		7.81	
Infected plants.....	258	24.29	320	27.21
Infected heads.....	1,002	9.23	1,108	12.06

That the application of manure and phosphate induced a more vigorous growth is shown by the fact that plants on the fertilized plot produced an average of 2.4 more heads per plant than those on the unfertilized plot. Associated with this increase in vegetative vigor was a reduction in the percentage of smutted plants from 27.21 to 24.29 and in the percentage of smutted heads from 12.06 to 9.23.

The results obtained in the greenhouse and field experiments conducted by the writer, and those obtained by Fromme (12), Hiltner and Lang (16), and Tieman (36), show that the vegetative vigor of wheat grown from seed infected with the loose-smut fungus has little influence on its susceptibility and is without practical significance in control.

SUMMARY AND CONCLUSIONS

Loose smut of wheat takes an estimated toll in the United States of over 10,000,000 bushels annually. Measures for controlling the disease rarely are applied, however, due chiefly (1) to the endemic nature of the disease and its ready escape from observation, (2) to the relative impracticability of all the known control measures, including the generally recommended modified hot-water treatment, and (3) to the fact that the latter generally reduces germination, retards emergence, and decreases yield. In view of this situation, the problem of controlling loose smut of wheat seems especially suited for solution by selecting or breeding resistant strains or varieties.

The life history of the causal organism (*Ustilago tritici*) and the previous investigations on the control of loose smut in wheat are briefly reviewed.

A comparative study was made of the anthesis of the susceptible Dawson (C. I. 6161) and the resistant Forward (C. I. 6691) wheats to determine a possible relation to resistance. No relation was found, the extent and duration of glume opening being practically the same for both varieties. The varietal-resistance studies, in which inoculum was artificially inserted within the flowers, showed further that resistance was not due to escape from inoculum. Observations indicate that a very early or very late blooming may enable a variety to escape infection provided the variety itself is free from loose smut.

The stage of anthesis in which wheat flowers were inoculated was a factor in the susceptibility of the plants to infection. Inoculation while the pollen was still immature caused higher infections than those when the pollen was ripe, but the inoculation of several highly re-

sistant varieties when the pollen was immature did not affect their resistance.

In the varietal resistance and susceptibility studies, 102 recognized varieties and 132 different lots, mostly pure-line selections of eastern wheats, were tested from one to three years. The flowers were inoculated by hand.

Pentad (C. I. 3322), the only durum wheat included in the experiment, was highly resistant. The three club wheats tested, Hybrid 128 (C. I. 4229), Jenkin (C. I. 5177), and Little Club (C. I. 4066), were strikingly susceptible, the latter two containing over 97 per cent of smutted plants and heads. In view of the fact that loose smut occurs but rarely in the Pacific Coast States, where these wheats are mostly grown, it seems probable that the low atmospheric humidity which generally prevails there when the wheats are in bloom plays an important rôle in preventing their infection.

In the common wheats the range of resistance ran from high susceptibility to apparent immunity. The highly resistant varieties were relatively few, but one or more were found in each of the four commercial groups except the white wheats. Susceptible strains and strains which thus far have been resistant were found in the varieties Fulcaster, Gipsy, Harvest Queen, and Red May. The following 13 varieties have been tested for two or three years and have proved to be highly resistant or immune under the conditions of the experiment: Bacska (C. I. 6156), Blackhull (C. I. 6251), Forward (C. I. 6691), Fulcaster (C. I. 3605), Hussar (C. I. 4843), Leap (C. I. 4823), Preston (C. I. 3081), Purplestraw (C. I. 1915), Ridit (C. I. 6703), Russian (C. I. 5737), Sol (C. I. 6009), Trumbull (C. I. 5657), and Wyandotte (C. I. 3549).

The pure-line selections of Hussar (Red Hussar C. I. 4843) and Ridit (Smutproof C. I. 6703) were highly resistant to and immune from loose smut, respectively. These same selections also have proved highly resistant to stinking smut.

Evidence was obtained indicating the presence of physiologic forms in *Ustilago tritici*.

No correlation was found between the hydrogen-ion values or the titratable-acid values of the juice of wheat plants and their ability to resist the invasion of *Ustilago tritici*.

More winterkilling occurred in plants grown from infected seed than in plants from uninfected seed, at Rosslyn, Va., and at Ithaca, N. Y.

Wide differences in the vegetative vigor of wheat plants grown from seed infected with the loose-smut fungus had little influence on their susceptibility.

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RELATION OF LEAF ACIDITY TO VIGOR IN WHEAT GROWN AT DIFFERENT TEMPERATURES¹

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INTRODUCTION

This investigation of the effect of temperature on leaf acidity was suggested by observations which indicated that any growth condition causing a decrease in the vegetative vigor of the wheat plant results in an increase in the acidity of its juice. For instance, winter wheats sown in the spring, which were so injured by unfavorable growth conditions that they failed to head, developed an abnormally high acid concentration (8).² These observations suggested that the acidity might vary consistently with the type of growth and so reflect the degree of adaptability of a variety to a given environment.

By growing both spring and winter varieties at each of three different temperatures, plants were obtained which differed greatly with respect to vigor and capacity for normal development. Comparative measurements of the acidity and moisture content of these plants at intervals during the growing period have given information bearing on the relation between the plant's morphological response to its environment and its chemical composition.

METHODS

Seed of the varieties Hard Federation (a spring wheat), Turkey (a hard red winter wheat), and Harvest Queen (a soft red winter wheat which is intermediate in its response to temperature) were sown on December 5, 1927, in each of three greenhouses at the Arlington Experiment Farm, Rosslyn, Va. These greenhouses were maintained at different temperatures, referred to as low, medium, and high, respectively. The benches on which the plants were grown were located in the same relative positions in each house so that light conditions were the same for all. The soil moisture was kept as uniform as possible throughout.

The temperatures were approximately controlled by hand regulation of the steam valves and ventilators. The air-temperature ranges were roughly 12°–18° C. in the low-temperature house, 20°–25° in the medium-temperature house, and 25°–30° in the high-temperature house. The corresponding soil temperatures were approximately

¹ Received for publication Mar. 19, 1929; issued September, 1929.

² Reference is made by number (italic) to "Literature cited," p. 349.

12°–15°, 18°–20°, and 23°–25°. At night the temperatures of both soil and air usually dropped to the lower limit of each range and sometimes below on cold nights. Until near the end of March the upper limits were exceeded only occasionally on warm afternoons. During April, however, only intermittent control of the low-temperature and medium-temperature houses was possible. The experiment was concluded early in April, therefore, when the plants were 18 weeks old.

The leaves of the plants selected for the determinations were ground to a pulp in a Nixtamal mill. The juice was expressed by squeezing it through cheesecloth. Its hydrogen-ion concentration and titratable acidity³ were determined electrometrically according to methods described previously (6). The specific gravity of each sample of juice was determined by means of a small pycnometer. In order to clarify the portion of juice used for this measurement, it was heated to 60° C. before filtering. The percentage of dry matter in the leaves was determined by drying samples to practically constant weight at 100° C.

In order to obviate the effects of diurnal changes in the composition of the plants, they were always cut at about 10 o'clock in the morning. The measurements were made as quickly as possible in the sequence of cutting, in order to minimize changes in the juice on standing.

The plan of sampling throughout most of the experiment was to select plants of one variety growing at each of the three different temperatures for the samples taken on any one day. The corresponding measurements for the other two varieties were made on the two following days, or as soon thereafter as possible. The final values, obtained when the plants were 18 weeks old, were determined in a different order. To emphasize at this time the comparative effects of each temperature on the three different types of wheat, plants of the three varieties growing at one temperature were taken each day, until the plants at all three temperatures were sampled.

Similar sampling plans were followed for the corresponding dry-weight determinations, although these were not always made on leaves cut on the same day as were the leaves for the juice samples.

RESULTS

Hard Federation, the spring wheat, grew vigorously and yielded well at both the low and the medium temperatures (12°–18° and 20°–25° C.) and was the only variety to produce heads at the high temperature (25°–30°). The heads produced at the high temperature were mostly sterile, however, and the plants were very much stunted. (Fig. 1.) Turkey, the winter wheat, grew normally at the low temperature, but it grew so poorly at the medium temperature that it produced only a few heads, and at the high temperature it did not develop beyond the tillering stage. Harvest Queen grew normally at the low temperature, but heading was retarded and irregular at the medium temperature. Its development was so inhibited by the high temperature that it produced no heads, many of the plants never progressing

³ The term "titratable acidity" is used to indicate the quantity of N/20 NaOH required to bring the reaction of 10 c. c. of juice to pH 8.3, the phenolphthalein end point.

beyond the tillering stage. Some reached an early shooting stage at the age of about 22 weeks, but none developed farther.

In Table 1 are given the hydrogen-ion, titratable-acid, and specific-gravity measurements and the dry-weight percentages for the leaves

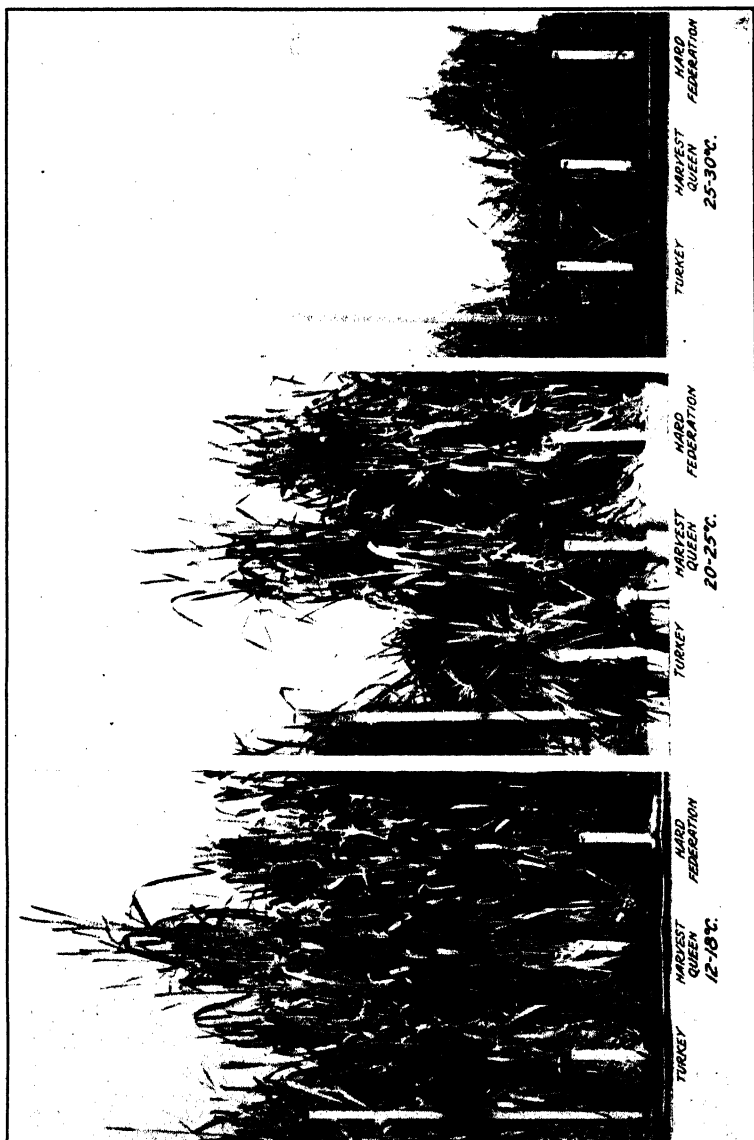


FIGURE 1.—Effects of three different temperature ranges on the growth of Hard Federation (a spring wheat), Turkey (a winter wheat), and Harvest Queen (a winter wheat which was somewhat intermediate in its temperature relations). Plants 22 weeks old

of representative plants of each lot, selected at intervals between the early tillering stage and the cessation of temperature control at the age of 18 weeks.

TABLE 1.—Comparative effects of different temperatures on the acidity and specific gravity of leaf juice and on the percentages of dry matter in the leaves of plants of certain spring and winter wheat varieties

[Roman type = tillering stage; italic = shooting stage; boldface = period of head formation]

Age (in weeks)	Spring wheat (Hard Federation)			Intermediate (Harvest Queen)			Winter wheat (Turkey)		
	12°-18° C.	20°-25° C.	25°-30° C.	12°-18° C.	20°-25° C.	25°-30° C.	12°-18° C.	20°-25° C.	25°-30° C.
pH values									
5	6.25	6.02	6.04	6.15	6.04	5.94	6.16	6.04	5.91
8	6.08	6.01	5.90	6.11	5.98	5.92	6.19	6.09	5.84
9	6.16	6.10	6.04	6.07	6.02	5.84	6.12	5.90	5.83
12	6.12	6.04	5.96	6.09	6.04	5.80	6.01	5.95	5.79
15-16	6.11	6.10	5.79	6.01	5.90	5.64	5.89	5.85	5.65
18	6.13	6.07	5.74	5.94	5.85	5.65	5.84	5.65	5.63
Titratable acidity (c. c. N/20 NaOH to neutralize 10 c. c. of juice)									
5	9.3	7.7	9.4	9.1	7.1	8.0	9.9	7.7	9.6
8	7.4	7.1	9.6	7.3	6.8	8.6	7.5	6.4	9.5
9	7.0	5.9	7.6	7.7	6.1	8.6	8.4	7.6	8.7
12	5.7	6.1	8.4	7.2	5.6	8.8	8.7	6.9	8.9
15-16	8.5	7.5	10.6	8.1	7.6	12.2	9.2	6.9	10.8
18	8.4	8.4	13.3	10.0	9.0	12.3	11.1	9.7	11.4
Specific gravity of juice									
5	1.0328	1.0290	1.0374	1.0313	1.0269	1.0320	1.0289	1.0263	1.0293
8	1.0295	1.0289	1.0334	1.0271	1.0267	1.0307	1.0305	1.0266	1.0326
9	1.0225	1.0213	1.0254	1.0233	1.0226	1.0271	1.0271	1.0251	1.0271
12	1.0253	1.0244	1.0302	1.0248	1.0223	1.0296	1.0331	1.0259	1.0311
15-16	1.0300	1.0289	1.0361	1.0278	1.0272	1.0293	1.0304	1.0269	1.0317
18	1.0278	1.0305	1.0391	1.0268	1.0285	1.0335	1.0292	1.0316	1.0335
Percentage of dry matter in leaves									
3*	12.53	10.84	13.69	12.45	10.19	12.93	13.88	11.28	13.50
5	15.10	13.96	16.62	14.54	12.21	15.09	13.84	12.40	14.48
8	13.65	12.57	15.50	12.67	12.08	15.79	14.02	12.81	14.42
9	12.43	12.27	14.12	11.65	10.85	13.13	14.21	12.94	13.80
12	12.75	12.40	15.61	12.33	12.11	13.91	15.60	12.16	14.62
15-16	18.23	18.67	20.03	17.50	16.26	18.71	14.81	14.74	14.62
18	18.08	18.30	24.47	16.64	16.37	15.97	17.32	14.82	15.05

* The dry-weight percentages at the age of 3 weeks were determined in connection with another experiment, but are included here because they were made on the same plants and under conditions comparable with those of the other measurements. No measurements on the juice were made at this time.

† Single determination; duplicate sample lost in drying.

DISCUSSION OF RESULTS

HYDROGEN-ION DETERMINATIONS

The pH values in Table 1 show the extent to which the hydrogen-ion concentration of the leaf juice of wheat is determined by the temperature at which the plants are grown. In each variety the lowest pH values were obtained for plants grown in the high-temperature house. The only exception was Hard Federation at the age of 5 weeks. Without exception, the highest pH values characterized plants grown in the low-temperature house.⁴

⁴ In a later experiment (1929) a lower temperature range (approximately 11° to 14° C.) was maintained in the low-temperature house by means of automatic control devices. The rate of development of seedlings growing at this temperature was distinctly slower than that of the low-temperature seedlings of the experiment reported in this paper. Their pH values were lower (between 5.8 and 5.9) than those of the more rapidly developing seedlings of the next higher temperature (approximately 21° to 24° C.), which had values near pH 6.0. The experiment was repeated with similar results. So it seems that, although the hydrogen-ion concentrations of the present experiment all varied directly with temperature—a relation resulting from the fact that the best development occurred throughout at the lowest temperature—a still lower temperature range, one so low as to cause severe retardation of the seedlings, increases their acidity above that of seedlings grown at a more favorable temperature. This finding constitutes further evidence of the close relation between the pH value and the vigor of growth.

It has been found (8) that the hydrogen-ion concentration of the juice of healthy wheat plants growing under greenhouse conditions remains relatively low throughout the vegetative period and increases during the maturation period, reaching a relatively high value by the soft-dough stage. In the present experiment only those plants which were highly vigorous (low-temperature Hard Federation and Harvest Queen and medium-temperature Hard Federation) maintained their original low hydrogen-ion concentration (near pH 6.0) throughout their vegetative period. The hydrogen-ion concentration of those plants which were somewhat less sturdy (medium-temperature Harvest Queen and low-temperature Turkey⁵) increased after about three months; and that of plants which were markedly stunted and retarded (high-temperature Turkey and Harvest Queen and medium-temperature Turkey) began to increase at an earlier stage. Thus external manifestations of the unfavorable effects of the high temperatures were found to be associated with evidence of an unbalanced metabolism.

There was an evident relationship between the course of these changes in hydrogen-ion concentration and the degree of adaptability of the variety to the particular temperature. For instance, the winter wheats, Turkey and Harvest Queen, showed the first and most severe injury at the high temperature. Their hydrogen-ion concentrations rose sooner, and eventually to higher values, than did those of the spring wheat, Hard Federation, which was less injured by this temperature.

The medium temperature brought out most clearly the differences in the climatic adaptability of the three varieties. Here Hard Federation grew vigorously and maintained a normally low hydrogen-ion concentration throughout its development, whereas Turkey developed slowly and was characterized, as signs of injury became apparent, by increasing hydrogen-ion concentrations. Harvest Queen was intermediate with respect to its ability to develop at this temperature, and in its late stages, when retardation became apparent, it was intermediate with respect to hydrogen-ion concentration.

TABLE 2.—*The correlation between the hydrogen-ion concentration of the expressed juice of wheat and the vigor of the plants at the age of 18 weeks; as shown by their general appearance, stage of development, and height*

Condition of plants	Variety and habit	Temperature	Stage of development	Approximate height (cm.)	pH
Group 1: Highly vigorous; normal development.	Hard Federation (spring).	Low	Past flowering	130	6.13
	do.	Medium	do.	105	6.07
	Harvest Queen (intermediate).	Low	Heading	125	5.94
Group 2: Moderately vigorous; irregular heading.	do.	Medium	Shooting	95	5.85
	Turkey (winter)	Low	do.	85	5.84
Group 3: Injured; stunted growth; sterile heads.	Hard Federation (spring).	High	Past flowering	65	5.74
Group 4: Severely injured; development arrested at an early vegetative stage.	Turkey (winter)	Medium	Shooting	65	5.65
	Harvest Queen (intermediate).	High	Tillering	45	5.65
	Turkey (winter)	do.	do.	30	5.63

⁵ The weaker growth of Turkey may have been partly due to shading, inasmuch as the adjacent rows of Hard Federation and Harvest Queen were taller throughout the experiment.

In Table 2 the pH values obtained at the age of 18 weeks, when the experiment was concluded, are grouped according to the relative degree of vigor of the plants at that time, as indicated by a comparison of their general appearance, stage of development, and height. In considering the data in this table in connection with the appearance of the plants shown by the photograph in Figure 1, taken four weeks later,⁶ it should be noted that the lesser vigor of the plants in Group 2 as compared with those in Group 1 was obvious not only from their retarded development but also from their irregular heading which gave them a ragged appearance.

Table 2 shows the degree of correlation between the hydrogen-ion concentration and the relative vigor of each different lot of plants. The most vigorous plants all had pH values between 5.94 and 6.13, and the most injured ones all had pH values between 5.63 and 5.65. Reference to Table 1 will show that the plants in the most severely injured group had lower pH values than the rest at each sampling period after about the first two months. These high acid concentrations were evidently indications of the abnormality which resulted eventually in inability to produce heads.

It appears, then, that the absolute magnitudes of the pH values⁷ reflected the physical condition of the plants. Values above pH 5.9 were obtained for healthy, vigorously growing plants regardless of variety. Values near 5.8 were obtained for moderately vigorous plants. Values as low as 5.65 were obtained only for slow-growing, extremely stunted plants.

There is considerable evidence that a slight increase in hydrogen-ion concentration often occurred before the adverse effects of an unfavorable environment could be ascertained by visual inspection. The plants with pH values as low as 5.9 in early stages of development were all considered healthy but they showed subsequently a diminution in vigor. Apparently, under the conditions of this experiment, a value near 5.9 during the vegetative period constituted a prediction of the subsequent appearance of symptoms of injury or retardation.

The conclusions of this experiment, in so far as they relate to absolute pH values, probably can not be extended to situations where the environmental complex is different. Light and soil conditions, as well as temperature, affect the hydrogen-ion concentration and determine the normal values for a given set of conditions (1, 4, 5, 10, 11, 15). Thus the hydrogen-ion concentrations obtained for some of the best field plants at the Arlington Experimental Farm were considerably higher than those for the vigorous greenhouse plants. Field plants of Harvest Queen, Shepherd, and Purplestraw had pH values of 5.74, 5.72, and 5.75, respectively, during the shooting stage, when the most vigorous greenhouse plants all had pH values above 6.0. However, the field plants were shorter than these best greenhouse plants, and their leaves were narrower and their culms less thick. Perhaps the tall, broad-leaved greenhouse plants, with their low concentrations of hydrogen ions, should be considered abnormally

⁶ The rapid elongation of the culms of Harvest Queen during these four weeks was responsible for the difference between the relative heights of the different lots of plants as given in Table 2 and as shown in Figure 1.

⁷ It should be noted that these absolute values are only significant, from the standpoint of vigor, during the period of vegetative development and up to the time during the maturation period when the normal increase in hydrogen-ion concentration begins.

vigorous. At any rate, their pH values consistently reflected the apparent difference in their vegetative development as compared to that of the field plants.

The persistent association of high hydrogen-ion concentration with lack of vigor recalls the fact that abnormal acidity of animal fluids also is associated with injury. Van Slyke in his studies of acidosis (16) found that the normal reaction of blood is between pH 7.3 and 7.5, and that values below 7.0 or above 7.8 are incompatible with life. It is not unreasonable to suppose that the plant also is very sensitive to changes in the reaction of its tissue fluids. Of course it is impossible to conclude that the high hydrogen-ion concentrations found in unhealthy plants have a causal association with injury. They may be merely incidental.

TITRATABLE ACIDITY, SPECIFIC GRAVITY, AND DRY-WEIGHT PERCENTAGES

Unlike the hydrogen-ion concentrations, the titratable-acidity measurements were not directly correlated with the temperatures at which the plants were grown. (Table 1.) The lowest values were generally obtained for the medium-temperature plants, which had also the lowest specific-gravity measurements and dry-weight percentages, up to the time of the final measurements when poor temperature control made comparisons uncertain. Although wheat is a low-temperature plant (2, 14, 17), these plants grew more rapidly than those at either the lower or the higher temperature during early stages of growth. Dickson (2) and Tottingham (14) also found that in early stages wheat grows more rapidly at temperatures above the optimum for growth at later stages and for maturation.

Reed (13) states, in connection with his conclusion that rapid vegetative growth is associated with high water intake and low sap concentration, that soil moisture seemed to be the determining factor. In the present experiment, the relatively high water content of the medium-temperature plants could not have been due to a higher soil moisture. In fact, the soil often was drier and rarely appreciably wetter than the soil in the other houses.

Under the conditions of these experiments, the titratable acidity of the juice usually varied directly with the specific gravity; i. e., with the total concentration of solutes. This conclusion is borne out by the fairly high correlation coefficient, 0.7314, which is obtained on considering all the titratable-acid and specific-gravity data as a group, regardless of the temperatures at which the plants were grown. The degree of correlation of these measurements is shown graphically in Figure 2.

The high-temperature plants generally had the highest titratable-acid concentrations as well as the highest specific-gravity measurements and the highest percentages of dry matter. In general it may be said that plants grown at temperatures so high that they are prevented from heading are characterized by higher acidity measurements and lower water content than are plants that develop normally. However, the titratable-acid measurement did not vary so consistently with the external appearance of the plants as did the hydrogen-ion concentration, which, by virtue of the buffer system, is independent of dilution within fairly wide limits (9).

The inhibiting effect of high temperature on the growth and maturation of wheat, especially of winter wheat, is in agreement with the observations of Dickson (2), Tottingham (14), Pojarkova (12), and others. Dickson (3) found that a change in the type of metabolism occurs at about those temperatures at which the change in morphological response occurs. He found a progressive decrease in sugars and dextrins in wheat grown at temperatures above 16° or 20° C. Tottingham (14) points out that temperature determines the type of metabolism by controlling the equilibrium between certain anabolic and catabolic reactions. He suggests that, since the rate of respiratory activity doubles between the relatively low and high temperatures of 15° and 25°, respectively, while the increase in rate of assimilation is appreciably less, such an increase in temperature should decrease the carbohydrate-protein ratio. His analyses show low-temperature plants

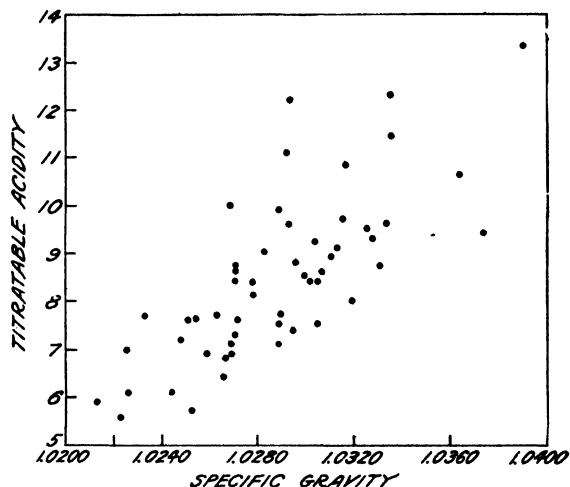


FIGURE 2.—Correlation between titratable acidity (c. c. N/20 NaOH) and specific gravity of wheat juice

to be characterized by a higher carbohydrate and protein content than are high-temperature plants.

It appears from the results of the present experiment that low-temperature plants are characterized also by lower hydrogen-ion concentrations than are high-temperature plants,⁸ and that the extent of the increase in acidity induced by high temperatures is indicative of the susceptibility of the individual variety to injury at the given temperature. In other experiments it has been observed that not only high temperature but any condition that appreciably lowers the vigor of the plant (6, 7, 8) causes an increase in the acidity of the juice. At least in the case of wheat and corn it has been found to be generally true that a high degree of vegetative vigor results in normally low acid concentrations, and, conversely, that poor growth is associated with abnormally high concentrations.

⁸ See footnote 4.

CONCLUSIONS

The concentration of hydrogen ions in leaf juice of Hard Federation, Harvest Queen, and Turkey wheats grown at temperatures of 12°-18°, 20°-25°, and 25°-30° C., respectively, was found to be lowest at the low temperature and highest at the high temperature.

The medium-temperature plants of all three varieties grew most rapidly at first, and almost without exception had the lowest titratable acidity, specific gravity, and dry-weight percentages throughout their vegetative stages, or as long as the temperature differences in the greenhouses could be maintained. The high-temperature plants generally had the highest titratable acidity, specific gravity, and dry-weight percentages.

The magnitudes of the titratable-acid values were closely correlated with those of the specific-gravity measurements at all three temperatures.

The pH value reflected the degree of adaptability of each variety to the different temperatures. Those plants which were best adapted, as shown by their vigorous growth and development, had pH values near 6.0 throughout the experiment. Those plants which were so injured that they failed to develop beyond the shooting stage developed much higher hydrogen-ion concentrations, extreme injury being accompanied by values near 5.6 while the plants were still in a vegetative stage.

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ADDITIONAL HOSTS OF *FUSARIUM OXYSPORUM* VAR. *MEDICAGINIS*¹

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INTRODUCTION

In recent publications^{2,3} the writer described a wilt disease of alfalfa caused by *Fusarium oxysporum* var. *medicaginis*, n. var. Since this fungus so closely resembles *F. oxysporum* morphologically and yet differs from it parasitically, it was thought that a further study of its host range should be made. With this in view, field and greenhouse experiments were conducted, the results of which are recorded in this paper.

GROWTH TESTS AT WEST POINT, MISS.

As the fungus is present in the soil of the Government experiment farm near West Point, Miss., it was thought desirable to grow different plants that might serve as hosts in this infested soil. H. L. Westover, agronomist in charge of the station, put at the writer's disposal one of the alfalfa plots in which the disease seemed to be most prevalent. Counts made of the plants from 3-yard-square sectors, one at each end and one at the center of this plot, showed that an average of 12½ per cent of the plants were infected. The crop of hay was removed and the ground was plowed and put into condition for planting. Two rod rows of each crop, located as far from each other as possible, were used in the test. Seeds of the following crops were planted on April 24, 1927. Acala cotton, Lone Star cotton, Early Black cowpea, Whip-poorwill cowpea, Peking soy bean, Wilson Five soy bean, Tom Watson watermelon, Lespedeza, crimson clover, hairy vetch, white sweet clover, yellow sweet clover, black medic, white clover, Horsford's Market Garden pea, Oregon red clover, and tomato.⁴

Observations were made on these plots throughout the season by T. F. Akers, superintendent of the experiment farm, who sent plants suspected of having the disease to the writer. The experiment was discontinued on September 1, 1927, at which time all of the plants were pulled and the roots split open to determine if there was any evidence of vascular browning. Isolations were made from all plants showing discoloration in the bundles. Two of the tomato plants developed wilt, but *Fusarium lycopersici* was obtained from them.

¹ Received for publication Feb. 28, 1929; issued September, 1929. These investigations were conducted in cooperation with the Kansas Agricultural Experiment Station. Paper No. 288 of the Department of Botany and Plant Pathology, Kansas State Agricultural College.

² WEIMER, J. L. A WILT DISEASE OF ALFALFA CAUSED BY *FUSARIUM* SP. *Phytopathology* 17: 337-338. 1927.

³ WEIMER, J. L. A WILT DISEASE OF ALFALFA CAUSED BY *FUSARIUM OXYSPORUM* VAR. *MEDICAGINIS*, N. VAR. *Jour. Agr. Research* 37: 419-433, illus. 1928.

⁴ Tomato plants purchased at the store; variety not known, but they were not resistant to *Fusarium lycopersici*.

Three of the red-clover plants showed typical vascular browning from which a species of *Fusarium* resembling *F. oxysporum* var. *medicaginis* was isolated. However, this fungus failed to infect alfalfa plants in subsequent trials. No *Fusarium* wilt was found in any of the other plants. Of the specimens sent by Akers, the alfalfa-wilt *Fusarium* was obtained only from hairy vetch. The infected plants were dying, and the stems showed some splitting and a little vascular browning. Isolations were made and a culture of what appeared to be *F. oxysporum* var. *medicaginis* was obtained. On one of his visits to West Point the writer found some garden peas and some common vetch plants which were dying and also showed evidence of vascular browning. Isolations were made from both the peas and the common vetch, and a fungus resembling *F. oxysporum* var. *medicaginis* in its cultural characters was obtained from each. These fungi, together with the one from hairy vetch, were later inoculated into alfalfa plants by inserting the spores and hyphae into wounds, and they produced 100, 100, and 90 per cent infection, respectively. The typical *Fusarium*-wilt symptoms were produced in the alfalfa plants, and the *Fusarium* was recovered in each case. This leaves little doubt that garden peas, common vetch, and hairy vetch, under certain conditions, may also serve as hosts for this species of *Fusarium*.

GROWTH TESTS AT MANHATTAN, KANS.

As a check on the experiments at West Point, Miss., similar tests were made at Manhattan, Kans. Here the soil was not infested with the fungus; hence the plants had to be inoculated. This was designed also as an alfalfa-variety test. Two rod rows of 16 different varieties of alfalfa as well as one row of each of the following plants were planted on a uniform piece of well-drained clay soil: Lespedeza, black medic, hairy vetch, white clover, yellow sweet clover, white sweet clover, Oregon red clover, crimson clover, *Vicia sativa*, Horsford's Market Garden pea, Midwest soy bean, Manchu soy bean, Trice cotton, Tom Watson watermelon, John Baer tomato, and Whippoorwill, Early Red, Early Buff, and Taylor cowpeas. In some cases one-half of one row and in other cases one-half of both rows of alfalfa were inoculated on June 7, 1927, by pouring a heavy spore suspension about the base of the plants. One-half of the plants of garden pea, soy bean, cotton, cowpea, and tomato were inoculated both by pouring a spore suspension about the plants and by inserting spores and hyphae into wounds in the tap root slightly below the surface of the soil. The remainder of the plants in each case were held uninoculated as controls.

The garden peas were pulled and examined on July 25. One plant which had been inoculated without wounding and three inoculated in wounds showed slight vascular browning. Isolations were made from three of the plants, and *Fusarium oxysporum* var. *medicaginis* was recovered from one of them, but not from the other two. This fungus produced wilt in alfalfa plants in a later experiment and was again recovered. There was no infection in any of the controls. These, together with the results obtained at West Point, show that the alfalfa-wilt fungus can cause a disease of garden peas. Only a comparatively small percentage of infection was obtained, and the vascular browning was rather slight in extent and seemed to be lim-

ited largely to the root. The plants infected remained small, and the leaves withered and died, those below dying first.

No infection was found in any of the other plants, except in one alfalfa plant of the Grimm variety. The fact that only one alfalfa plant became infected indicates that conditions were not very suitable for infection. However, not all of the perennial plants were pulled, the presence or absence of infection being judged by the top symptoms.

A further test of the susceptibility of different varieties of alfalfa was made by reinoculating the same lots of plants in June, 1928.

The fungus used was grown on sterilized oats in 1-liter flasks, so that a large quantity of inoculum was available. Ten plants of each variety were inoculated by inserting spores and hyphae beneath the bark of the taproot near the crown. Other plants were inoculated by pouring the infested oats into a trench about the crowns of the plants and covering them with soil. The number of plants inoculated varied with the variety. In some cases only about 10 were available, whereas in others probably as many as 100 were used.

On September 7, 1928, all of the inoculated plants were dug, and the roots were cut open and examined carefully for the presence of vascular browning. All of the diseased plants were sectioned and studied microscopically, and abundant hyphae were found in the tracheal tubes. No isolations were made, as typical symptoms together with the presence of abundant hyphae in the ducts were considered sufficient evidence of the presence of the wilt disease. Only five plants in all were infected. Two of these were of the Grimm, two of the Hardigan, and one of the Turkestan variety.

Since only one plant was infected in 1927 and five in 1928, no conclusions can be drawn with respect to the comparative susceptibility of the varieties used. It seems apparent that some conditions at Manhattan, Kans., are not suitable for the infection of alfalfa by the wilt fungus.

SUMMARY

Hairy and common vetch and garden peas have been found to be susceptible to *Fusarium oxysporum* var. *medicaginis*, the alfalfa wilt-producing fungus.

An attempt was made to study the comparative susceptibility of 16 varieties of alfalfa under field conditions at Manhattan, Kans., but the plants that became infected were so few in number that no conclusions could be drawn.

VITAMIN CONTENT OF HONEY AND HONEYCOMB¹

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INTRODUCTION

Honey has been considered a valuable food since earliest times. Consisting as it does of a mixture of dextrose and levulose it is easily digested, and this fact may account in part for the good results obtained when it is used in the diet, especially in infant feeding. Since honey can be used to advantage in the diet of infants the question arose as to whether it might not be a source of some or all of the vitamins. A review of the literature revealed the fact that very little work had been done in the way of determining quantitatively the vitamin content of honeys.

REVIEW OF LITERATURE

Dutcher (3)² determined the vitamin B content of honey obtained while basswood and white clover were in full bloom, using pigeons in his work. The tests were made by absorbing the vitamin of the honey on Lloyd's reagent and feeding amounts equivalent to 45 gm. of honey. Nectar was tested in the same manner. Dutcher concluded that the strained honey contained a negligible amount of vitamin B and that there was little evidence of its presence in nectar.

In 1919 Bachman (1) found that 25 c. c. of a strained honey added to 75 c. c. of water and used in Nagel's solution did not furnish the vitamin necessary for the growth of yeast.

Faber (4) in 1920 made a study of the antiscorbutic value of a white-sage comb honey which was extracted before using. Guinea pigs were used, and all of them exhibited characteristic scurvy symptoms when fed a solution of 1 part of honey to 15 parts of water, which was later increased to 1 part of honey to 5 parts of water. The quantity of honey consumed ranged from 0.88 to 5.58 c. c. of honey per 100 gm. of initial body weight. Faber concluded that it was "probable" that honey contained no antiscorbutic vitamin.

Hawk, Smith, and Bergeim (5) determined the vitamin A, B, and C content of blended honey, white-clover honey, and honeycomb. For vitamin B their method consisted in feeding three groups of rats, respectively, (1) a diet free from vitamin B; (2) one in which blended honey replaced part of the starch; and (3) one in which white-clover honey replaced part of the starch. At the end of four weeks the diets were changed. Group 1 was divided and half the rats were given blended honey and the other half white-clover honey. After another two weeks all were given milk. From the results obtained, Hawk and his associates concluded that there was a small amount of vitamin B present in these honeys. Following a similar procedure for the

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² Reference is made by number (italic) to "Literature cited," p. 366.

vitamin A determination, they found that strained honey contained no vitamin A, whereas a definite but minimal amount was present in the comb honey. For the vitamin C test they fed three groups of guinea pigs in the same way; that is, they gave one group the scorbutic diet, a second group the same diet with blended honey to replace a part of the starch, and a third group the same diet with clover honey to replace the starch. All developed scurvy within two weeks, showing that the honeys contained no vitamin C.

In 1922 Luttinger (6) gave a general report of his findings on the use of honey in infant feeding in which he states that he found vitamins A, B, C present in 82 per cent of the honey examined. No experimental evidence was presented to bear out this statement.

Scheunert, Schieblich, and Schwanebeck (8), in 1923, examined three samples of honey for vitamins A, B, C, and concluded that none of the samples contained vitamins.

Caillas (2), in 1925, reported work done with pigeons which seemed to show that fresh honey contained vitamin B. The number of birds used, however, was too small to make the results very convincing.

DESCRIPTION OF HONEYS TESTED

Since it was out of the question to make an exhaustive examination of honeys of all the principal floral sources, three samples representing the extremes of color variation were chosen for investigation. None of the honeys had been heated as is often the case with extracted honey. Honey No. 1 was a white-clover honey from Grover Hill, Ohio. This was in a granular state when received. Honey No. 2 was a buckwheat honey, very dark in color, produced near Varysburg, N. Y. Honey No. 3 was a light-colored white-clover honey from Middlebury, Vt. It was drained from the comb and the comb was pressed as free from adhering honey as possible and was also used in feeding tests.

EXPERIMENTAL DATA

VITAMIN A DETERMINATIONS

The method used for vitamin A determinations was essentially that of Sherman and Munsell (11) with a few modifications.

The basal diet consisted of casein (purified), 18 per cent; starch, 67 per cent; brewery yeast, 10 per cent; Osborne and Mendel salts, 4 per cent; table salt, 1 per cent. The diet was irradiated with the light from a mercury vapor quartz lamp to insure an abundance of vitamin D. The rats were fed the vitamin-A-free diet until stationary or declining weight and appearance of symptoms due to vitamin A deficiency indicated that their body stores of vitamin A were depleted. As soon as the rats were in a suitable condition to be used for tests they were weighed and placed in individual cages. A weighed amount of the vitamin-A-free food was given to each rat and the honey was fed as a daily supplement to this diet.

Honeys No. 1 and No. 2 were fed in amounts of 1, 2, and 3 gm. per day. The plan of feeding daily portions of honey to the rats required a great deal of time. For this reason honey No. 3 and the honeycomb were incorporated in the basal diet in place of 30 per cent of the starch. In each litter one or more animals were designated as controls and received only the basal diet during the test period. The test period was continued for eight weeks, or until it was terminated by

the death of the rat. If the rat did not live out the eight weeks the last recorded weight is that of the dead rat. Autopsies were performed on all animals to determine whether the gross pathological lesions shown by animals confined to a vitamin-A-free diet were present. Table 1 gives the weights and survival periods of the rats used for these tests. Curves showing the changes in weight made by averaging results from the groups of test animals are presented in Figure 1.

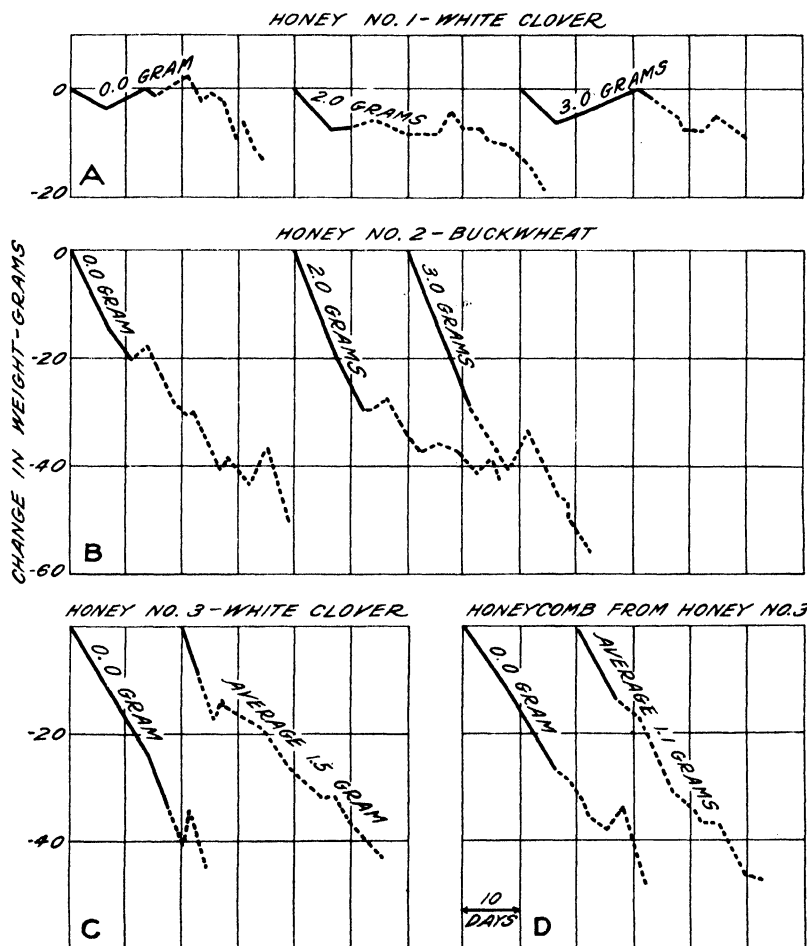


FIGURE 1.—Curves showing changes in weight made during the test period by groups of rats fed honey or honeycomb as the sole source of vitamin A. Each curve is the average result of several tests. The amount of honey or honeycomb received by each rat six times per week is indicated on each curve. The change in weight for the group is represented by a solid line to the point where the death of the first animal occurred. The broken line represents the averages for the surviving animals until all had died.

None of the rats receiving the honey or honeycomb lived out the full eight weeks of the test period, nor did they live on an average any longer than the control rats. In all cases the rats fed honey and honeycomb exhibited as severe pathological lesions as those that received no honey in addition to the basal diet.

WHITE-CLOVER HONEY (NO. 1)

Quantity of honey fed per rat per day, 6 days per week (grams)	Rat No.	Weight of rats at age of 4 weeks	Weight of rats when feeding of honey was begun	Weight of rats at end of successive weeks of test period								Period of survival
				1	2	3	4	5	6	7	8	
		Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams
0	907	54	67	65	68	74	67	52				91
	922	50	65	62	63	71	54	50				84
	923	48	58	52	60	56	59	42				87
	937	51	66	64	68	78	77	54				84
	968	61	80	83	98	86	71					74
	975	54	71	68	88	85	77	60				83
	993	59	88	92	85	84	77	66				79
	996	52	77	70	74	70	66	50				79
1110	36	80	69	56	53						57	
1123	45	85	78	75	76	58					59	
Average												77.7
2.0	908	53	70	64	63	70	64	54				91
	921	46	55	53	51	57	53	45				91
	935	58	79	71	75	83	77	69				82
	972	56	61	53	49							59
	974	54	70	67	82	83	79	66	55	51		93
	994	57	83	77	77	60	61	57				79
	1107	64	116	95	95	75						63
	1120	45	92	84	80	79	60					61
Average											77.4	
3.0	924	44	52	45	50	49	45					77
	925	42	50	45	48	52	44	36				84
	933	62	76	64	67	86	92	82	67			89
	934	52	66	58	60	67	62	49				84
	971	57	67	62	81	84	70	59				81
	995	55	86	84	77	76	60	58				78
	1119	46	95	88	83	78	77	71				67
	Average											80.0

BUCKWHEAT HONEY (NO. 2)

0	1366	55	134	123	109	84						55
	1373	48	111	91	91	68	66					56
	1391	55	130	110	105	104	88	75				70
	1396	52	120	96	98	96	65					65
	1404	57	136	125	127	112	109	100	85			74
	1405	58	124	113	121	105	77					63
	1415	59	139	123	100							48
	1420	54	111	109	95	73						56
Average												60.9
2.0	1368	50	126	109	103	81	69					59
	1372	53	117	102	90	82	74	74				66
	1390	59	147	120	93	88						54
	1393	55	115	103	95	94	98	76	72			74
	1403	61	157	125	115	95	94					57
	1407	56	118	97	109	100	82	67				67
	1417	52	119	104	80							49
	Average											60.9
3.0	1365	56	139	125	92	90						42
	1371	54	120	97	83	65	72	68				63
	1389	59	137	126	95	81						69
	1392	49	126	94	99	94	80	68				59
	1402	63	153	135	107	89						62
	1406	67	126	96	103	90	77					54
	1414	61	132	121	111	91						58
	1418	51	123	106	81							48
Average											55.6	

TABLE 1.—Weight records of rats fed honey and honeycomb as the sole source of vitamin A—Continued

LIGHT-COLORED WHITE-CLOVER HONEY (NO. 3)

Quantity of honey fed per rat per day, 6 days per week (grams)	Rat No.	Weight of rats at age of 4 weeks	Weight of rats when feeding of honey was begun	Weight of rats at end of successive weeks of test period								Period of survival
				1	2	3	4	5	6	7	8	
		Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams
0.....	2908	58	114	102	93	80						65
	3086	57	131	108	95	79						65
	3089	52	114	105	93	80	69					69
	3092	49	110	105	92	70						65
Average.....												66.0
Average, 1.5 (30 per cent of diet).....	2903	63	137	101								54
	2904	62	120	113	109	89						67
	2906	57	134	94								54
	2907	58	98	87								51
	2909	55	118	109	96	94	76					76
	2910	54	115	85	95	85	84	72				83
	2911	50	106	82								54
	3085	57	125	118	98	94	80					70
	3087	50	104	87	84							53
	3088	56	123	105	108	90	92					72
	3090	51	105	95	90	78	76	64				76
	3091	50	117	100	87	80						66
	3093	40	100	94	91	83	63					72
Average.....												65.2

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0.....	3128	58	114	107	107	83	72					65
	3129	57	117	110	88	74						62
	3131	54	112	100	94	86	88	69				74
	3141	70	153	144	132	116	97					68
	3142	64	136	128	119	101	100	83				75
	3145	57	111	105	102	75	65					66
	3444	59	145	126	97	90						58
	3445	57	124	101	90	84						60
	3448	52	110	105	89	85	69					70
	3450	44	102	94	84	67	66					64
Average.....												66.2
Average, 1.1 (20 per cent of diet).....	3125	61	134	112	100	86	84					64
	3127	59	122	110	107	86	72					70
	3130	56	114	105	92	78	74					67
	3132	47	106	96	82	63						59
	3144	60	130	119	96	95	84	76				73
	3143	54	111	107	94	74						62
	3146	57	117	110	92		85	70				76
	3446	55	120	80								49
	3447	52	114	95	86							53
	3449	52	103	100	85	79	65					67
Average.....												64.0

These results indicate that no one of these three samples of honey nor the honeycomb contained an amount of vitamin A that could be detected by the method used for measuring this factor.

VITAMIN B DETERMINATIONS

The determination of the vitamin B content of the three samples of honey was completed before the multiple nature of vitamin B had been generally recognized. The method used was that of Sherman and Spohn (12), which makes no distinction between the two vitamin B factors. All rats were kept in cages having raised screen bottoms and were given a basal diet of casein (purified) 18 per cent, starch 68 per cent, butterfat 8 per cent, cod liver oil 2 per cent, Osborne and Mendel salts 4 per cent. Honey No. 1 was fed in amounts of

TABLE 2.—Weight records of rats fed honey and honeycomb as the sole source of vitamin B—Continued

BUCKWHEAT HONEY (NO. 2)

Quantity of honey fed per rat per day, 6 days per week (grams)	Rat No.	Weight of rats at age of 4 weeks	Weight of rats at end of successive weeks of test period								Period of survival
			1	2	3	4	5	6	7	8	
			Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	
0.....	1961	45	41	41	36	30	26	-----	-----	-----	34
	1968	41	40	36	31	25	-----	-----	-----	-----	26
	1972	41	39	37	33	27	-----	-----	-----	-----	28
	1985	36	34	31	27	25	-----	-----	-----	-----	24
	1989	32	32	29	26	21	19	-----	-----	-----	30
Average.....											28.4
Average, 0.7 (30 per cent of diet).	1962	44	41	42	38	33	27	26	-----	-----	38
	1963	42	41	38	35	31	25	-----	-----	-----	34
	1964	48	45	44	41	35	29	27	-----	-----	38
	1965	46	41	37	35	31	28	28	-----	-----	39
	1966	45	40	39	35	30	26	-----	-----	-----	33
	1967	42	40	38	35	27	25	-----	-----	-----	31
	1969	32	30	28	27	23	20	-----	-----	-----	35
	1970	44	39	37	33	25	22	-----	-----	-----	32
	1971	44	41	39	35	29	24	24	-----	-----	36
	1973	41	38	36	33	29	25	25	-----	-----	36
	1974	39	38	34	31	26	22	-----	-----	-----	33
	1984	35	30	31	27	23	21	-----	-----	-----	30
	1986	34	33	34	30	25	21	-----	-----	-----	30
	1987	33	30	30	27	21	-----	-----	-----	-----	28
	1988	32	32	30	28	24	23	-----	-----	-----	32
	1990	37	35	32	29	28	23	-----	-----	-----	35
	1991	35	31	31	27	23	21	-----	-----	-----	31
	1992	34	31	32	29	24	21	-----	-----	-----	30
Average.....											33.4

LIGHT COLORED WHITE-CLOVER HONEY (NO. 3)

0.....	3010	49	49	45	41	34	30	-----	-----	-----	31
	3014	45	46	43	38	32	27	-----	-----	-----	31
	3015	45	45	42	36	31	29	-----	-----	-----	30
	3072	52	50	48	40	36	32	-----	-----	-----	32
	3075	52	48	44	43	38	33	28	-----	-----	38
	3079	45	41	38	38	30	29	-----	-----	-----	29
	3081	45	41	37	36	30	26	-----	-----	-----	31
Average.....											31.7
Average, 0.9 (30 per cent of diet).	3009	50	52	49	44	38	33	-----	-----	-----	31
	3011	48	50	49	41	34	33	-----	-----	-----	30
	3012	47	47	45	40	33	30	-----	-----	-----	30
	3013	49	50	47	42	35	31	-----	-----	-----	30
	3016	45	47	44	40	32	30	-----	-----	-----	30
	3071	55	56	51	46	39	36	-----	-----	-----	35
	3073	52	52	50	44	38	35	-----	-----	-----	31
	3074	53	49	49	43	35	32	-----	-----	-----	30
	3077	48	46	44	42	38	33	-----	-----	-----	31
	3078	48	43	42	38	33	29	-----	-----	-----	31
	3080	45	40	39	34	31	27	-----	-----	-----	31
Average.....											30.9

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Average, 0.5 (20 per cent of diet).	3173	50	48	45	39	34	31	29	-----	-----	40
	3174	50	48	45	38	33	29	-----	-----	-----	34
	3175	47	43	42	35	32	28	-----	-----	-----	34
	3176	45	44	41	36	30	28	-----	-----	-----	33
	3177	48	45	42	35	31	28	-----	-----	-----	33
	3178	47	45	41	34	31	-----	-----	-----	-----	27
	3179	46	44	39	34	31	-----	-----	-----	-----	28
Average.....											32.7

BUCKWHEAT HONEY (NO. 2)

70	414	428	252	29	Moderate	++	+++	+	+	+	+	12
71	420	420	224	25	Severe	++	+++	+	+	+	+	17
67	419	420	238	21	Moderate	++	+++	+	+	+	+	14
68	419	420	229	23	Moderate	++	+++	+	+	+	+	9
69	427	466	228	23	Moderate	++	+++	+	+	+	+	17
64	413	443	243	32	Severe	++	+++	+	+	+	+	16
63	413	413	249	21	do.	++	+++	+	+	+	+	16
63	413	413	249	21	do.	++	+++	+	+	+	+	16
Average				28.3								14.4

LIGHT-COLORED WHITE-CLOVER HONEY (NO. 3)

75	442	464	263	36	Severe	++	+++	+	+	+	+	18
73	443	472	245	33	do.	++	+++	+	+	+	+	19
76	453	461	291	29	do.	++	+++	+	+	+	+	17
74	437	453	261	25	do.	++	+++	+	+	+	+	15
Average				30.8								17.3

• Intensity of scurvy symptoms is indicated by plus signs each having an arbitrary value of 1; minus signs indicate absence of symptoms.

No one of the honey samples nor the honeycomb enabled the rats to live any longer or to make any greater gains in weight than the rats that received no additions to the basal diet. These results show that the honeys and honeycomb examined contained no vitamin B.

VITAMIN C DETERMINATIONS

The method used to determine the vitamin C content of honey was that described by Sherman, LaMer, and Campbell (10). No tests were made on the honeycomb. The basal diet described by Sherman (9) consisted of skim-milk powder heated at 110° C. for 4 hours, 30 per cent; a mixture of equal parts commercial rolled oats and wheat bran, 59 per cent; butterfat, 10 per cent; table salt, 1 per cent. The guinea pigs were somewhat heavier at the beginning of the test period than the standard animal described by Sherman. Some difficulty had been experienced in other work in getting the smaller guinea pigs to eat the basal diet satisfactorily. In these tests the preliminary period was continued until it was ascertained beyond a doubt that the guinea pigs would eat the basal diet.

The honey was fed apart from the basal diet, and the test period was continued in each case until the guinea pig died. Honey is not relished by guinea pigs, and the feeding required a great deal of time and patience. The intake of honey was calculated as grams per day per 300 gm. of initial body weight. Table 3 gives the results of the feeding tests. For purposes of comparison each plus (+) under autopsy findings has been given a value of 1 and these have been totaled for each animal.

On an average the guinea pigs that had the honey did not live appreciably longer than those that had none. Before death and at autopsy all showed symptoms of scurvy that were as severe as the symptoms shown by the control animals. It is evident from these results that the three samples of honey examined contained no appreciable amounts of vitamin C.

VITAMIN D DETERMINATION

The method for testing for vitamin D is identical with that described in a previous paper (7). Young rats approximately 60 grams in weight were placed on the Steenbock low phosphorus diet consisting of yellow corn, 76 per cent; wheat gluten, 20 per cent; calcium carbonate, 3 per cent; and sodium chloride, 1 per cent, for 21 days, at which time they showed evidences of a rachitic condition. The honey was then fed during a test period of not less than 6 nor more than 15 days. At the end of the test period a line test was made according to the method described by McCollum (15). While this method may not be entirely satisfactory for quantitatively measuring vitamin D it seemed to us to be preferable to any other method worked out to date.

The three honeys tested were incorporated in the basal diet to the amount of 30 per cent, 10 per cent, and 20 per cent, respectively, and the honeycomb as 10 per cent. In each litter of rats used for testing honey No. 1 there was one or more control rats which were given 0.5 per cent cod-liver oil during the test period instead of honey. This plan of having positive controls was not considered necessary in the tests with the other samples.

Summaries of the results of these tests are given in Table 4. As all of the line tests with the honeys and the honeycomb were negative, the results with individual rats are not given. X-ray photographs were also made of the rats used in these determinations. These pictures check the line tests made on the corresponding rats in that all showed severe rickets. From these results it would seem that none of the honeys examined nor the honeycomb contained any amount of vitamin D that would cause calcium deposition in rats which had been maintained for 21 days on the Steenbock low-phosphorus-yellow-corn diet.

TABLE 4.—Summary of tests made to determine the vitamin D content of honey and honeycomb as compared with a cod-liver oil supplement

WHITE-CLOVER HONEY (NO. 1)

Test food in the diet (per cent)	Duration of test period	Number of cases	Average intake of honey per 100 gm. of rat per day	Average value of line test
	<i>Days</i>		<i>Grams</i>	
0.....	0	11	-----	0
	11	5	-----	0
	13	6	-----	0
	15	1	-----	0
30.0.....	11	13	2.36	0
	12	2	1.98	0
	13	13	2.39	0
	15	1	2.49	0

COD-LIVER OIL

0.5.....	9	15	0.04	4
	11	2	.04	4

BUCKWHEAT HONEY (NO. 2)

0.....	0	4	-----	0
	13	2	-----	0
	15	4	-----	0
	11	2	0.70	0
10.0.....	13	3	.67	0
	15	3	.70	0
20.0.....	13	2	1.45	0
	15	2	1.42	0

LIGHT-COLORED WHITE-CLOVER HONEY (NO. 3)

0.....	0	3	-----	0
	15	3	-----	0
20.0.....	13	12	1.49	0
	15	12	1.50	0

HONEYCOMB

0.....	0	1	-----	0
	13	1	-----	0
10.0.....	9	3	0.80	0
	13	3	.79	0

CONCLUSION

The three samples of honey examined were produced in widely separated localities and represented extremes of color variation. No detectable amounts of vitamins A, B, C, or D were found in any of the honeys or in the honeycomb.

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CORRELATED INHERITANCE IN A WHEAT CROSS BETWEEN FEDERATION AND A HYBRID OF SEVIER × DICKLOW¹

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INTRODUCTION

Several wheat characters, such as culm length, spike density, and awn classes, have been shown to exhibit rather complicated inheritance. This paper reports a study of the inheritance and of the correlated inheritance of these and some other plant characters in a cross between Federation and a hybrid of Dicklow × Sevier (III C-18).

LITERATURE

The literature which applies to inheritance of the wheat characters herein reported has recently been rather thoroughly reviewed by Clark (3),² by Hayes and Garber (5), and by Stewart (7). For this reason only references which bear directly on the particular problems under discussion are cited.

DESCRIPTION OF PARENTS

Federation has been rapidly growing in importance in Utah and Idaho as an irrigated wheat. This is largely due to its high-yielding ability and to its habit of not lodging under irrigation. Its straw is short and stiff, and its glumes are dark bronze in color. It is classed as an awnless wheat (4), yet short-tip awns or beaks are usually present.

Different characters were measured on the 37 parent rows which were grown along with the F₃ progenies. The mean values for these are given below:

Length of longest culm to base of spike.....	cm.....	75.34
Spike density—length of one rachis internode.....	mm.....	5.02
Length of awn.....	do.....	5.05
Neck thickness.....	do.....	2.40
Culms per plant.....	number.....	10.13

III C-18 is one of the high-yielding hybrid strains selected by Stewart at the Utah experiment station from a Sevier × Dicklow cross. It inherits to some extent, at least, the weak straw of its Sevier parent. Its glumes are white, and it is fully awned.

Measurements of plant characters of III C-18 in the 37 parent rows gave the following mean values:

Length of longest culm to base of spike.....	cm.....	91.96
Spike density—length of one rachis internode.....	mm.....	2.62
Awn length.....	do.....	73.07
Neck thickness.....	do.....	2.34
Culms per plant.....	number.....	9.46

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² Reference is made by number (italic) to "Literature cited," p. 392.

"The author is indebted to Dr. H. K. Hayes, of the Minnesota Station, for helpful criticisms in studying the data and arranging the manuscript. Dr. Fred Griffiee of the Maine Station has also made several valuable suggestions."

These measurements show the culm length of III C-18 to be about 17 cm. greater than that of Federation. The spike is nearly twice as dense; the awns have considerable length. In neck thickness and in number of culms per plant the parents are almost alike.

EXPERIMENTAL PROCEDURE

The cross between a pure line of Federation and the pure line III C-18 was made in 1924 at Logan, Utah. The F_1 plants were grown in 1925, and the F_2 families in 1926. One of the most vigorous of these families was chosen to continue the experiment. The F_2 family chosen contained 354 plants. The plants were divided according to awn appearance into four groups, designated as awn class 1, 2, 3, and 4, or merely as awns 1, 2, 3, and 4. The plants in the group of awn class 1 had practically no awns at all, though most of them bore short beaks. At the apex of the spike there was an occasional short awn. Considerable care was taken to have this group correspond in awn development to that of the Federation parent. Plants belonging to awn class 4 were fully awned to correspond to the awn development of the other parent III C-18. The plants in awn class 2 bore intermediate awns with the principal awn development in the apical part of the spike. It was the intention to make this group correspond in awn development with that of the F_1 plants. The plants in awn class 3 were likewise intermediate in their awn development. The apical awns, however, were considerably longer than those of awn class 2, and short awns were found quite generally all the way down the spike. In awn class 2, on the other hand, the awns were limited largely to the apical third of the spike, with a few awn points below, but even these were almost entirely in the upper half of the spike.

Plants were separated into two classes for glume color, bronze and white. Spike density was obtained by measuring 10 internodes on a leading spike of each plant. The measurement was taken in the middle of the spike so as to avoid the internodes of irregular length both at the base and at the apex. The length of the longest culm was measured to the base of the spike. Awn length was obtained by measuring the length of one of the longest awns near the apex of each spike. The neck thickness was obtained by measuring with calipers the diameter of the culm in the thinnest place just below the spike. Care was taken not to crush the straw.

The data were so taken and recorded that all those from a given plant could easily be traced to that plant. This permitted the study of correlation.

Grains from each F_2 plant seeded an F_3 progeny row in 1927. Forty to fifty kernels spaced 3 inches apart were sown in each F_3 row except when an F_2 plant furnished fewer kernels than this. The rows were spaced 1 foot apart.

Since each F_2 plant seeded one F_3 progeny row, it was possible to use the breeding behavior of the F_3 progenies as the basis for classifying the F_2 plants. This method is definitely superior to the method of classifying the F_2 material, especially when studying complicated characters and characters which exhibit intermediate inheritance.

After each tenth progeny row the two parental varieties, Federation and III C-18, were sown side by side. These parental rows

were spaced and seeded in the same manner and at the same time as were the progeny rows. In all, there were 37 parental pairs. This procedure made it possible to study progeny characters in connection with parental characters.

When the grain was ripe each F_3 row was carefully harvested by pulling individually the plants in the row. The plants of each row were bundled, tied, and tagged. During the winter months each plant from each F_3 progeny and from each parental row was studied in the laboratory in the manner previously described.

The usual number of plants in each progeny ranged from 25 to 40. In some progenies there were fewer than this and in many there were 40 or more. In each row of the 37 pairs of parental rows there was a similar number of plants. Measurements and classifications were made on the basis of what the genetic material contained. No theory of inheritance was considered until all data were taken and tabulated.

EXPERIMENTAL RESULTS AND THEIR INTERPRETATION

After all the data were secured and recorded, studies were made of those concerned with each individual character and then with correlations between characters. Data regarding color of glume and also regarding awn classes could be readily grouped into homozygous and heterozygous progenies for study of inheritance of individual characters. The characters awn length, length of the longest culm, number of culms, spike density, and neck thickness involved a definite figure for each plant in an F_3 progeny. A mean value for each character measured was calculated, together with standard deviations and coefficients of variability. Studies of the comparative size of these constants showed whether it was possible to segregate the progenies into homozygous and heterozygous groups, or whether segregation could not be established. When the correlation studies were made the mean values of the F_3 progenies were the figures used, the mean of an F_3 progeny being regarded as more accurate than an individual figure from a single F_2 plant.

INHERITANCE OF INDIVIDUAL CHARACTERS

Inheritance studies were made of the following individual characters: Glume color, awn classes, awn length, length of longest culm, number of culms, and spike density.

GLUME COLOR

Eighty-two F_3 progenies were homozygous for bronze glumes, 176 were heterozygous for color of glumes, and 96 were homozygous for white glumes. These numbers suggest the 1:2:1 ratio which has been previously observed by several other workers. There is probably a single-factor difference for color of glumes. The goodness of fit on this hypothesis is shown in Table 1. $\chi^2=1.1186$ and $P=0.5782$, which is a good fit.

TABLE 1.—Goodness of fit of three groups of F_3 progenies for glume color, compared with a 1 : 2 : 1 ratio

(Grown in 1927 at Logan, Utah)

Progeny group	Calculated value (C)	Observed value (O)	O - C	(O - C) ²	$\frac{(O - C)^2}{C}$
Homozygous bronze.....	88.5	82	-6.5	42.25	0.4774
Heterozygous.....	177.0	176	-1.0	1.00	.0056
Homozygous white.....	88.5	96	+7.5	56.25	.6356

$$\chi^2 = 1.1186. \quad P = 0.5782.$$

AWN CLASSES

Figure 1 shows typical heads for each of four homozygous awn classes. Figures 2 to 5 show homozygous F_3 progenies for awn classes 1, 2, 3, and 4. Besides these homozygous classes there were five segregating classes of progenies, namely, those segregating for awn classes 1 and 2; those for awn classes 1, 2, and 3; those for awn classes 1, 2, 3, and 4; those for awn classes 2, 3, and 4; and those for awn classes 3 and 4.

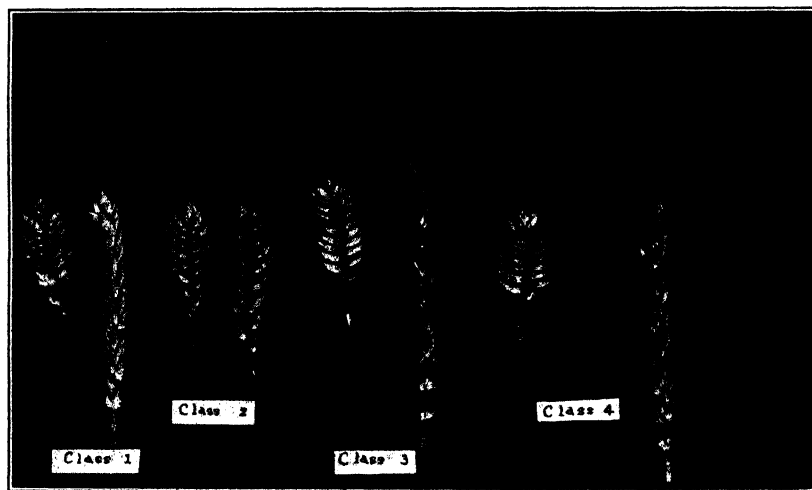


FIGURE 1.—Type of awns found in the four awn classes of Federation \times III C-18 wheat that are true breeding in the F_3 generation. In every case there is a somewhat greater awn development on the longer lax spikes. These classes are not purely theoretical; they were decided upon after a careful study of the F_3 progenies

There were, therefore, nine genotype classes into which the F_3 progenies were arranged in the following numbers:

	Progenies
1. Homozygous awn 4.....	23
2. Segregating awn 3, 4.....	38
3. Segregating awn 2, 3, 4.....	43
4. Segregating awn 1, 2, 3, 4.....	81
5. Homozygous awn 3.....	24
6. Segregating awn 1, 2, 3.....	46
7. Homozygous awn.....	24
8. Segregating awn 1, 2.....	50
9. Homozygous awn 1.....	25

These numbers suggest a ratio of 1:2:2:4:1:2:1:2:1, which would theoretically be obtained when 2-factor differences segregate independently.

Stewart (7) has suggested two linked factors with 35 per cent crossing over to explain the awn-class inheritance in Sevier \times Federation

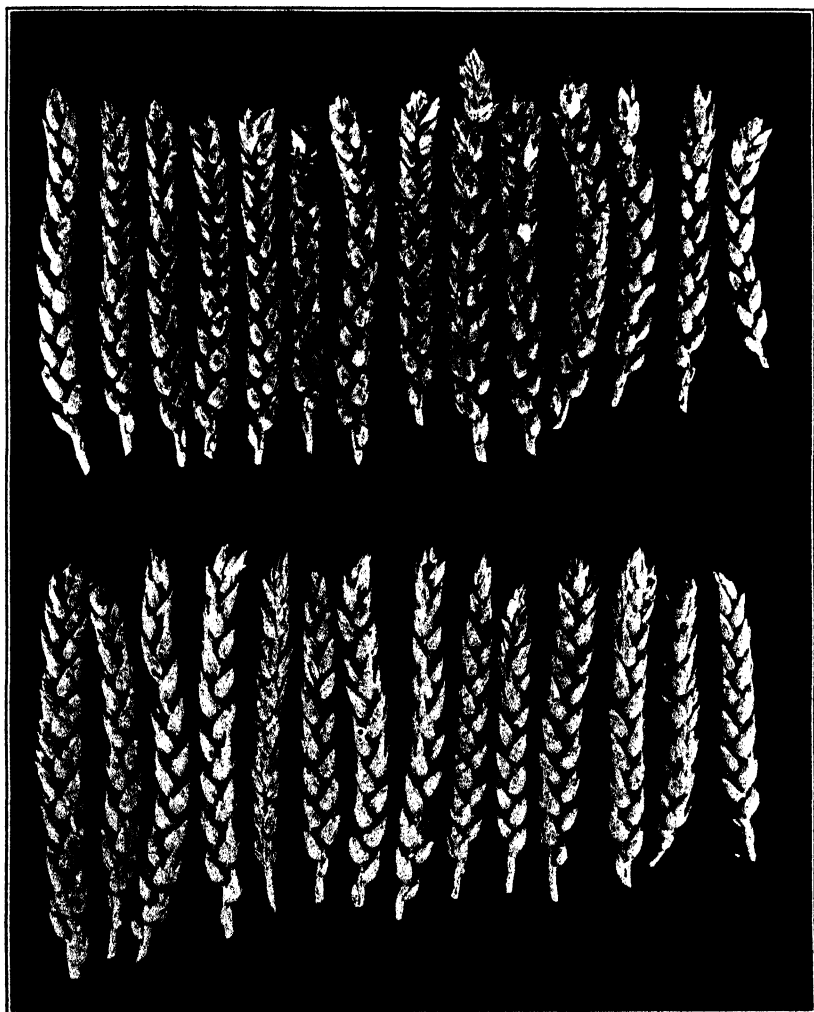


FIGURE 2.—One head from each plant of an F_2 progeny of Federation \times III C-18 wheat breeding true for awn class 1. These plants are not really awnless but have a few short awn points. The Federation parent showed a similar range in awn points

crosses and in a cross between a Sevier \times Dicklow hybrid (G 149) and Federation (8).

The awn-class inheritance in the Federation \times III C-18 can be explained on a 2-factor difference only where independent segregation rather than linkage occurs, such as the Howards found in a cross

some years ago (6). This suggests that the hybrids III C-18 and G 149 both derived from Federation \times Sevier crosses and both fully awned have different genetic constitutions for awn classes. One

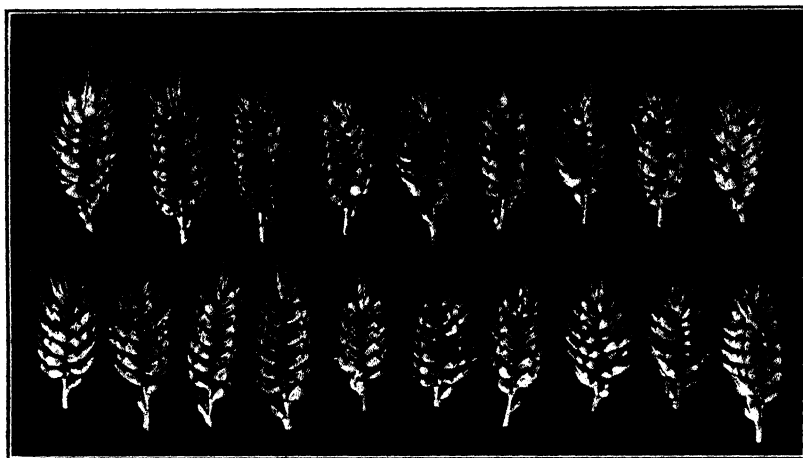


FIGURE 3. Representative heads from about half the plants of an F_3 progeny of Federation \times III C-18 wheat breeding true for awn class 2; the entire range is shown. These spikes bear short awns, but they are limited to the upper spikelets



FIGURE 4.—Representative heads from plants of an F_3 progeny of Federation \times III C-18 wheat breeding true for awn class 3. While some of these awns are quite long, none of them attain full length. The upper spikelets bear rather long awns while the middle and lower spikelets bear well-developed short awns and awn points

explanation which might be offered for this difference is that the Sevier variety of wheat is a composite of rather variable lines. One Sevier pure line when crossed with Dicklow may have given the strain which behaves like G 149, while another Sevier pure line crossed

with Dicklow may have given the III C-18 strain which behaves differently.

Since Federation \times III C-18 shows no linkage, the assumption is here made that at least one of the awn factors in III C-18 is different

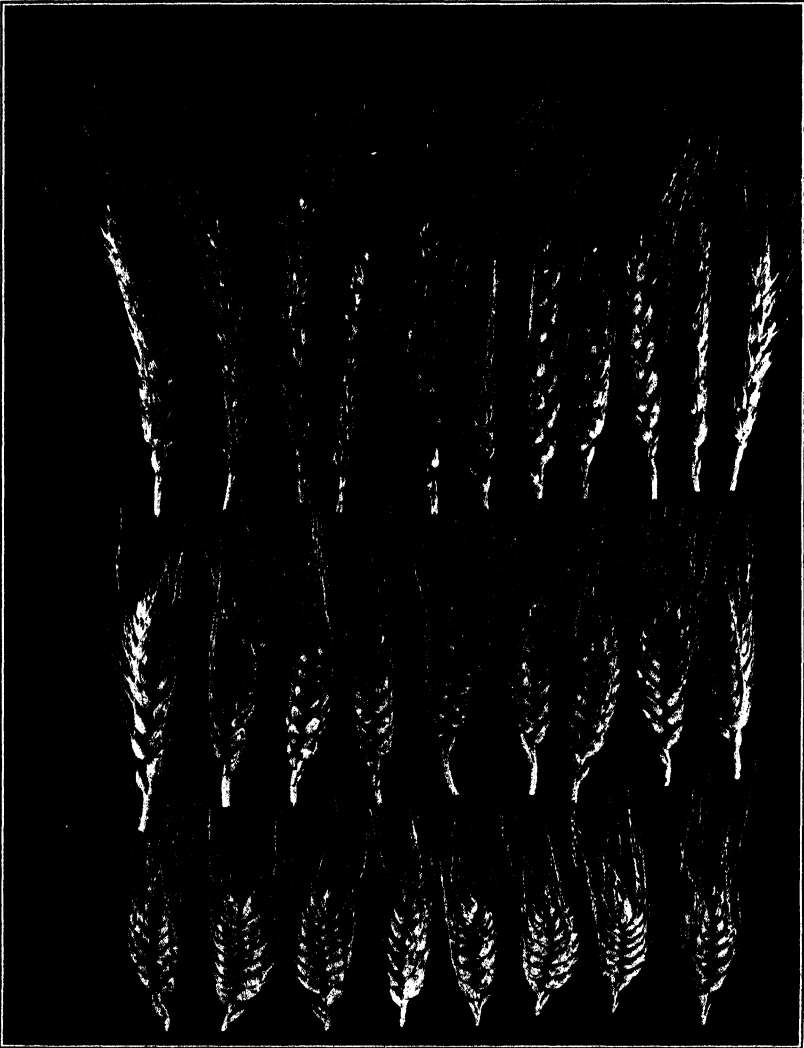


FIGURE 5.—Representative heads from plants of an F_2 progeny of Federation \times III C-18 wheat breeding true for full-length awns of class 4. Long awns are borne on all spikelets except those at the very base of the spike. The lax spikes have the longer awns

from that in G 149. The factors designated for the Federation \times G 149 cross (\mathcal{L}) were A (a) and T (t). This use of the dominant factors to indicate the presence of awns does not follow Biffen (1) and others, who have regarded lack of awns as the dominant condition.

When the F_1 condition is as nearly intermediate as in the case of awns in wheat, there seems no real reason for not using the dominant to represent the presence of the positive character. For the Federation \times III C-18 cross, let it be supposed that the factor A is the same as in the above cross but that T is not operative and is designated as tt , but that the factor B acts the same as T except that it is not linked with A . The awn classes would then be designated as follows:





Awn 4 = $AA\ tt\ BB$ 
 Awn 3 = $AA\ tt\ bb$ 
 Awn 2 = $aa\ tt\ BB$ 
 Awn 1 = $aa\ tt\ bb$ 

TABLE 2.— F_2 awn-class genotypes, their expected proportions on an independent segregation basis, and their expected breeding behavior

No.	Genotype	Expected proportions	Expected breeding behavior
1	$AA\ tt\ BB$	1	Breed true for awn class 4.
2	$AA\ tt\ Bb$	2	Segregate for awn classes 3, 4.
3	$Aa\ tt\ BB$	2	Segregate for awn classes 2, 3, 4.
4	$Aa\ tt\ Bb$	4	Segregate for awn classes 1, 2, 3, 4.
5	$AA\ tt\ bb$	1	Breed true for awn class 3.
6	$Aa\ tt\ bb$	2	Segregate for awn classes 1, 2, 3.
7	$aa\ tt\ BB$	1	Breed true for awn class 2.
8	$aa\ tt\ Bb$	2	Segregate for awn classes 1, 2.
9	$aa\ tt\ bb$	1	Breed true for awn class 1.

Table 2 shows the nine F_2 awn-class genotypes, their expected ratio, and their breeding behavior based on the above assumption. The goodness of fit between the observed data and the expected frequencies is given in Table 3. On the basis of nine segregating classes, $\chi^2 = 3.0961$ and $P = 0.9270$, which is an extremely good fit. A worse fit due to chance alone is to be expected in 93 out of 100 cases.

TABLE 3.—Goodness of fit of nine awn-genotype classes of F_3 progenies when compared with a 1 : 2 : 2 : 4 : 1 : 2 : 1 : 2 : 1 ratio which would theoretically be obtained when a 2-factor difference segregates independently

(Grown in 1927 at Logan, Utah)

Calculated value (C)	Observed value (O)	$O - C$	$(O - C)^2$	$\frac{(O - C)^2}{C}$
22.125	23	+0.875	0.7656	0.0346
44.250	38	-6.250	39.0625	.8828
44.250	43	-1.250	1.5625	.0353
88.500	81	-7.500	56.2500	.6356
22.125	24	+1.875	3.5156	.1589
44.250	46	+1.750	3.0625	.0692
22.125	24	+1.875	3.5156	.1589
44.250	50	+5.750	33.0625	.7472
22.125	25	+2.875	8.2656	.3736

$\chi^2 = 3.0961$. $P = 0.9270$.

It is interesting to note the resulting numbers in the four awn classes when the F_2 population was classified. The 354 F_2 plants were classified in 1926-27 before planting time just as were the F_3 progenies a year later. The numbers obtained for each of the awn-class groups were as follows:

Awn 4 = 49 F_2 plants
 Awn 3 = 35 F_2 plants
 Awn 2 = 106 F_2 plants
 Awn 1 = 164 F_2 plants

These data when compared with the F_3 data indicate that the F_2 plants which will breed true in F_3 may resemble rather closely other F_2 plants which will segregate in the F_3 generation. In handling data of this sort it is much more accurate to classify the F_2 genotypes on the basis of their F_3 breeding behavior than on the basis of the appearance of the F_2 plants.

AWN LENGTH

When the data on the F_3 progenies were first being secured it was thought to be unnecessary to measure the short-tip awns found on awn class 1 plants, but after about 90 of the progenies had been worked it was decided that this figure would be useful. The No. 1 awns were measured for the remainder of the progenies. Only the progenies in which all awn classes present were measured were used in making calculations of awn-length data. Since there was a fair representation in each awn-class group after eliminating the progenies in which the No. 1 awns were not measured, it is believed that the tables and calculations represent the material fairly accurately.

The mean awn length of the various awn classes together with their standard deviations and coefficients of variability are shown in Table 4.

TABLE 4.—Mean length of the awns on progenies belonging to true-breeding awn classes 1, 2, 3, and 4 and to five segregating groups, together with the standard deviation (S. D.) and coefficient of variability (C.V.) for each awn-class group

[Grown in 1927 at Logan, Utah]

Type of progeny	Mean awn length	Standard deviation	Coefficient of variability
True breeding for class:	<i>Mm.</i>	<i>Mm.</i>	<i>Per cent</i>
1	3.47	2.17	62.54
2	15.51	4.43	28.56
3	31.30	7.70	24.60
4	66.48	8.40	12.64
Segregating for classes:			
1, 2	7.33	5.52	75.31
1, 2, 3	19.05	12.92	67.82
1, 2, 3, 4	25.83	19.74	76.42
2, 3, 4	37.20	19.56	52.58
3, 4	48.06	16.36	34.04

TABLE 5.—Awn-length classes of the means of parental rows and of F_2 progenies arranged according to coefficients-of-variability (C. V.) classes of the individual rows of each parent and of the 9 awn-class-genotype groups of the F_3 progenies

[Grown in 1927 at Logan, Utah]

Parent or progeny	Number of plants in awn-length classes (millimeters)																		Total	C. V. classes
	2.5	7.5	12.5	17.5	22.5	27.5	32.5	37.5	42.5	47.5	52.5	57.5	62.5	67.5	72.5	77.5	82.5			
Federation	{ 2 7 1	{ 4 9 1																6 16 2	40.00 60.00 80.00	
Total or mean	10	14																24	58.13	
III C-18	{													4	23 1	8	1	36 1	10.00 20.00	
Total or mean														4	24	8	1	37	11.56	

TABLE 5.—Awn-length classes of the means of parental rows and of F_2 progenies arranged according to coefficients-of-variability (C. V.) classes of the individual rows of each parent and of the 9 awn-class-genotype groups of the F_2 progenies—Continued

Parent or progeny	Number of plants in awn-length classes (millimeters)																		Total	C. V. classes
	2.5	7.5	12.5	17.5	22.5	27.5	32.5	37.5	42.5	47.5	52.5	57.5	62.5	67.5	72.5	77.5	82.5			
Homozygous for awn class 1	{ 1 6 1 1	1																1 7 1 1	40.00 60.00 80.00 100.00	
Total or mean	9	1																10	62.54	
Segregating for awn classes 1 and 2	{ 1 5 14 2 1	1 1																2 5 15 5 1	40.00 60.00 80.00 100.00 120.00	
Total or mean	4	22	2															28	75.31	
Homozygous for awn class 2	{		7 2	10 4														17 6	20.00 40.00	
Total or mean			9	14														23	28.56	
Segregating for awn classes, 1, 2, and 3	{			1 3 4 2	1 1 4													2 9 11 2	40.00 60.00 80.00 100.00 120.00 140.00	
Total or mean			14	8	2													24	67.82	
Segregating for awn classes 1, 2, 3, and 4	{					1 1 9 6 3	2 8 4 2	1 3 8 2	2 3 1									1 3 29 12 3	40.00 60.00 80.00 100.00 120.00	
Total or mean				18	12	12	5	1										48	96.42	
Homozygous for awn class 3	{				1 3	7 3	7 3	2 4	4									21 3	20.00 40.00	
Total or mean					1	10	7	2	4									24	24.60	
Segregating for awn classes 2, 3 and 4	{						4 3 3 1	1 3 9 1	4 4 5 1	1 1 1								2 12 25 3	20.00 40.00 60.00	
Total or mean					1	3	11	14	9	3	1							42	52.58	
Segregating for awn classes 3 and 4	{						1 6	3 9 1	4 3 10	1 10	4 5	5 1						9 28 1	20.00 40.00 60.00	
Total or mean							1	4	7	11	9	5	1					38	34.04	
Homozygous for awn class 4	{								1		1	4	5	4	1	5	2	22 1	10.00 20.00	
Total or mean									1		1	4	5	4	1	5	2	23	12.64	

Table 5 shows the arrangement in awn-length classes and coefficient-of-variability classes of the nine awn-class genotypes. Standard deviation and coefficient-of-variability constants were both used in studying awn length. In the homozygous awn 1 group and in the awnless parent groups standard deviation seemed better to represent the true condition of the material on account of the very small mean awn length which unduly accentuated the size of the coefficients of variability. Usually the coefficient of variability is

the constant to use, and the one which was used for the classes shown in Table 5. It merely meets a mechanical difficulty in the case of the group ordinarily spoken of as awnless.

As shown in Table 6, homozygous-awn classes, except awn class 1, show a small range in coefficients-of-variability classes and also in mean coefficients of variability.

TABLE 6.—Range of the coefficients of variability (C. V.) and mean coefficient of variability of awn length in the parent rows in the four homozygous awn classes and in the segregating awn classes

[Grown in 1927 at Logan, Utah]

Rows or progenies	Range in C. V.	Mean C. V.
Parental rows:		
Federation.....	39.8 to 70.7	58.13
III C-18.....	6.7 to 20.7	11.56
True breeding for—		
Awn class 1.....	42.7 to 103.0	62.54
2.....	17.9 to 33.4	28.56
3.....	18.7 to 32.0	24.60
4.....	7.7 to 25.6	12.64
Segregating for—		
Awn classes 1, 2.....	43.2 to 116.2	75.31
1, 2, 3.....	39.9 to 133.9	67.82
1, 2, 3, 4.....	45.2 to 123.2	76.42
2, 3, 4.....	23.8 to 84.8	52.58
3, 4.....	26.6 to 53.2	34.04

The segregating awn classes all show a greater range and a higher mean coefficient of variability than do the homozygous classes, except the awn 1 group, which has been discussed. It might be expected that the longer-awned classes would show a greater range and higher mean standard deviation but a smaller mean coefficient of variability than do the shorter-awned classes. This is borne out by the figures.

The higher coefficients of variability (C. V.) in segregating classes tend to show that eye classification of awn classes was fairly accurate. The coefficients of variability are from two to three times as great in the segregating classes as in the corresponding true-breeding classes.

In awn length the range of homozygous awn 1 progenies practically covers the range of the Federation parent, and the awn 4 progenies that of the III C-18 parent. The awn 1 progeny range is from 2.35 ± 0.96 to 5.39 ± 2.46 mm. Federation ranges from 3.56 ± 1.38 to 6.62 ± 2.77 mm. The awn 4 progenies range from 42.52 ± 7.35 to 80.40 ± 4.89 mm. in awn length, while the III C-18 rows range from 67.20 ± 9.30 to 84.50 ± 3.84 mm.

The homozygous awn 2 progenies range from 11.28 ± 1.59 to 19.68 ± 3.94 mm. in awn length. The homozygous awn 3 range is from 22.12 ± 3.71 to 42.15 ± 6.65 mm.

LENGTH OF LONGEST CULM

There was considerable difference between the lengths of the longest culm of the two parent plants. When the means of the 37 parental rows were averaged, the following figures were obtained: Federation=75.34 cm. and III C-18=91.96 cm. The III C-18 parent is 16.62 cm. greater in culm length than is the Federation

parent. With this considerable difference between the parents, it could be expected that there would be a segregation in the F_2 generation and that there would be a tendency for the F_3 progenies to inherit the culm length of their respective F_2 parents.

TABLE 7.—Culm-length classes of the means of Federation and III C-18 parental rows and of the F_3 progenies

[Grown in 1927 at Logan, Utah]

Parent or progeny	Number of plants in culm-length classes (centimeters)														Total
	56	60	64	68	72	76	80	84	88	92	96	100	104	108	
Federation.....	1	---	1	3	8	11	9	3	1	---	---	---	---	---	37
III C-18.....	---	---	---	---	1	1	1	2	6	13	8	4	---	1	37
F_3 progenies.....	---	1	3	10	24	35	67	68	43	46	22	27	7	1	354

Table 7 shows the range in mean culm length for the parental rows and for the F_3 progenies. The parental classes overlap to some extent, but the shortest Federation row is four classes below the shortest III C-18 row, and the longest III C-18 row is five classes above the longest Federation row. Out of the 354 F_3 progenies the longest III C-18 row is practically recovered in one progeny. The longest III C-18 = 109.0 ± 3.95 cm. and the longest F_3 progeny = 107.75 ± 4.18 cm. The shortest Federation row is also practically recovered in one F_3 progeny. The shortest Federation row is 57.62 ± 3.72 cm. in culm length and the shortest F_3 progeny is 59.20 ± 4.40 . The range of the progenies, then, essentially covers the range of the parents, and the distribution resembles somewhat that of a normal curve. (Fig. 6.) This study indicated segregation of culm length but did not establish the nature of culm-length inheritance.

The culm length of the F_2 plants was correlated with the mean culm length of their respective F_3 progenies. A correlation coefficient of $+0.4316 \pm 0.0291$ was obtained. The constant is 14.8 times its probable error. This significant correlation obtained between the F_2 plants and their F_3 progenies indicates that segregation had occurred and that culm-length inheritance is displayed in this cross. This study did not find a means of separating the segregating groups.

NUMBER OF CULMS

The two parents average essentially the same number of culms per plant (Federation = 10.13 ± 1.37 and III C-18 = 9.46 ± 1.45). Correlation coefficients were calculated between number of culms in F_2 and F_3 and between all other plant characters studied. In every case the probable error was almost as large as its constant. In this cross, then, there seems to be no measurable segregation or inheritance in stooling ability as indicated by number of culms.

SPIKE DENSITY

As previously stated, the F_3 breeding behavior was used as the basis for determining the spike-density inheritance. The mean length of 10 rachis internodes of each F_3 progeny and of each parent

row was calculated. The coefficients of variability (C. V.) were also calculated and used as the basis for separating homozygous progenies from heterozygous. Table 8 shows the mean spike-density classes and the coefficient-of-variability (C. V.) classes of the parent rows and of the three groups of F_3 progenies, homozygous dense, heterozygous, and homozygous lax. Figure 7 shows the curves plotted for these same groups. Figure 8 illustrates the range in spike densities.

TABLE 8.—Mean spike-density and coefficient-of-variability (C. V.) classes of Federation and III C-18 wheat, and of the three groups of F_3 progenies of their cross—homozygous dense, heterozygous, and homozygous lax ^a

[Grown in 1927 at Logan, Utah]

Parent or progeny	Spike-density classes															Total	C. V. classes
	18	22	26	30	34	38	42	46	50	54	58	62	66				
Federation	{									1						1	4.00
									12	5						17	8.00
									4	12	2					18	12.00
									1							1	16.00
Total or mean								5	24	8						37	9.81
III C-18	{			15	1											16	8.00
				18	1											19	12.00
				2												2	16.00
Total or mean			35	2												37	10.59
Homozygous dense	{	2														2	4.00
		22	11													33	8.00
		16	22													38	12.00
		2	4	1												7	16.00
			1													1	20.00
Total or mean		42	38	1												81	10.70
Heterozygous	{			1	2			1								4	28.00
				4	5	3		1								13	32.00
			1	5	10	8	6									30	36.00
			1	11	20	19	4	1								56	40.00
			1	8	16	9	4									38	44.00
				3	8	8	2									21	48.00
					3	1	1									5	52.00
				1												1	56.00
				1				1								2	60.00
Total or mean		3	30	63	50	20	4									170	40.91
Homozygous	{							2	8	18	25	7	2			1	4.00
								2	5	18	8	5			1	62	8.00
											1					39	12.00
																1	16.00
Total or mean							4	13	37	34	12	2	1	103		9.56	

^a The vertical columns represent spike-density classes of the means of the parental rows and of F_3 progenies grouped horizontally by major groups into homozygous dense, heterozygous, and homozygous lax. The horizontal lines within each major group represent coefficients-of-variability (C. V.) classes. The mean coefficient of variability for each group is shown at the bottom of each group at the extreme right. The figures at the bottom of each group, on the "Total" line, are sums of the rows or progenies in that spike-density group. The figures in the second vertical column from the right (Total) are the sums of rows or progenies in that group falling within the coefficient-of-variability class designated.

The coefficients of variability of the Federation parent rows range from 7.47 to 14.65 per cent, with a mean of 9.81 per cent. The coefficients of variability of the III C-18 parent rows range from 4.9 to 14.14 per cent, with a mean of 10.59 per cent.

For the homozygous-dense F_3 progenies the range in coefficients of variability was from 4.78 to 18.12 per cent, with a mean of 10.7 per cent.

The coefficient-of-variability range in the homozygous-lax group was from 5.67 per cent to 15.16 per cent, with a mean of 9.56. Every progeny is clearly homozygous, as shown by its low coefficient of variability.

For the heterozygous F_3 progenies the range in coefficients of variability was from 27.42 to 61.28 per cent, with a mean of 40.91 per cent. Though there was one doubtful progeny, the actual range may be considered to be from 27 to 61 per cent. All these progenies have coefficients of variability sufficiently high to mark them clearly as heterozygous, whereas all the homozygous dense progenies have coefficients of variability sufficiently low to permit their ready classification as homozygous.

One progeny of only 13 plants gave a coefficient of variability of 17.19 per cent. This coefficient of variability indicates immediately that the progeny is true breeding. An examination of the plants themselves led to doubt in this respect. The lengths in millimeters of the

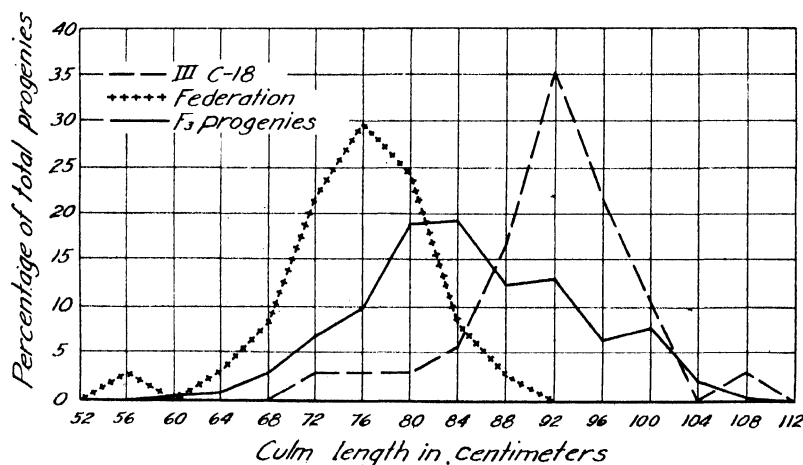


FIGURE 6.—Culm-length distribution curves of Federation and III C-18 parents and F_3 progenies; the entire culm-length range of both parents was recovered in the F_3 progenies.

10 spikelet internodes on the 13 plants were 20, 24, 24, 27, 27, 29, 30, 30, 30, 33, 33, 35, and 40. None of the definitely true-breeding dense progenies show a range of 20 mm. in length of 10 spikelet internodes. The presence of just one very dense-spiked plant and just one long intermediate leaves the coefficient of variability small. This case illustrates the importance of having 30 or 40 plants in the progenies. When this progeny is regarded as being true breeding it is the only one in which the spike density of a progeny falls definitely within the range of the more compact parent, III C-18. In Figure 7 it is shown considerably toward the lax side from any other true-breeding dense progeny. The writers are far from sure that this progeny was not heterozygous. Since it was not continued in F_4 , this was not definitely ascertained.

The means of the coefficients of variability of the two homozygous groups are nearly the same as the means of the parents. The range is also approximately the same. The heterozygous group has a much

higher mean and also a much wider range in coefficients of variability. Except for the one questionable progeny already discussed, the least variable heterozygous progeny is fully 50 per cent more variable than the most variable homozygous progeny, either of the dense or of the lax group. Such wide gaps establish the groups in clear-cut fashion.

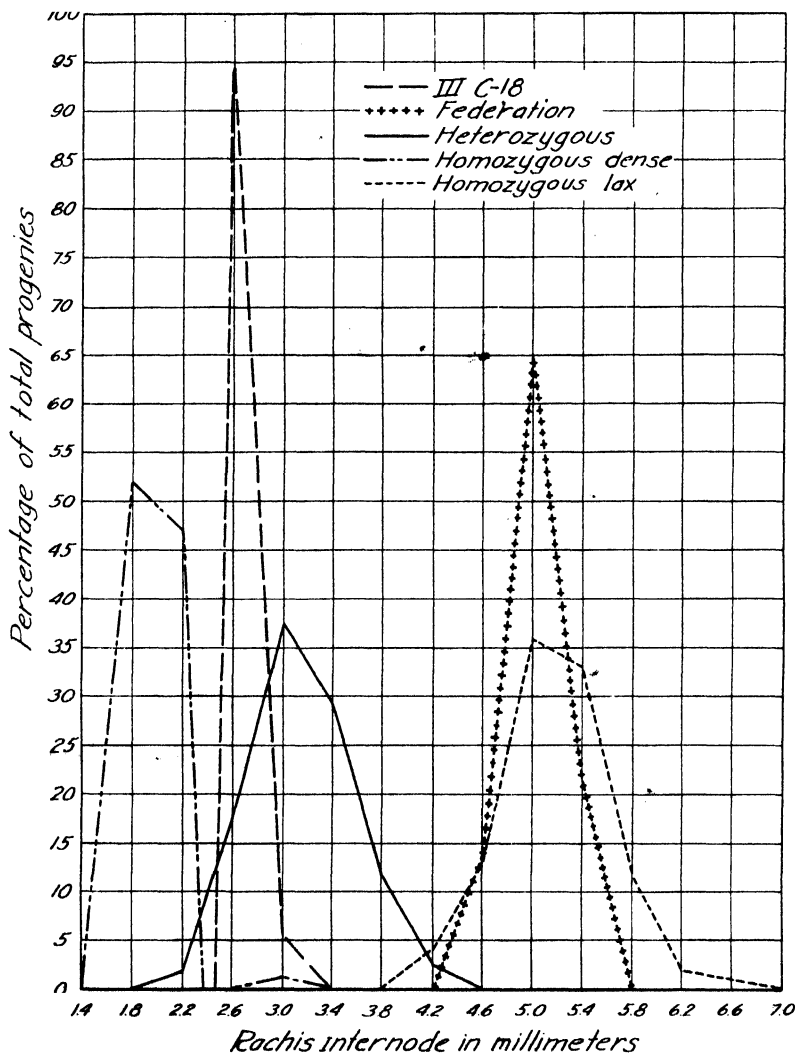


FIGURE 7.—Spike-density curves of Federation and III C-18 parents, and of three F_3 progeny groups—homozygous dense, heterozygous, and homozygous lax. Except for one progeny of doubtful breeding behavior the homozygous-dense progenies are all more dense than the more dense parent, III C-18

The range in spike density of the clearly homozygous-dense group of F_3 progenies is from 1.63 ± 0.10 to 2.37 ± 0.23 mm. per rachis internode. The most dense of the III C-18 parent rows was 2.41 ± 0.22 mm., which, it will be observed, is less dense (but not statistically

so) than the least dense of the homozygous-dense progenies. The mean density of the doubtful progeny was 29.38 ± 3.41 mm., which is definitely within the range of the more dense parent. The head density of III C-18 was not recovered except possibly in these two progenies, and perhaps not here. This is rather a peculiar situation which has occurred before in spike-density inheritance in crosses involving Sevier and Federation and is as yet unexplained.

The spike-density range in the homozygous-lax progenies seems to extend somewhat beyond the range of the Federation parent, though this is far from certain. Federation ranged from 4.66 ± 0.42 to 5.41 ± 0.18 mm. per rachis internode, while the homozygous-lax progenies range from 4.34 ± 0.33 to 6.40 ± 0.49 mm. The difference between 5.41 ± 0.18 and 6.40 ± 0.49 is less than twice the probable error.

The heterozygous progenies are intermediate between the two homozygous groups and overlap them in but a few cases, giving in the main a distinct trimodal curve.

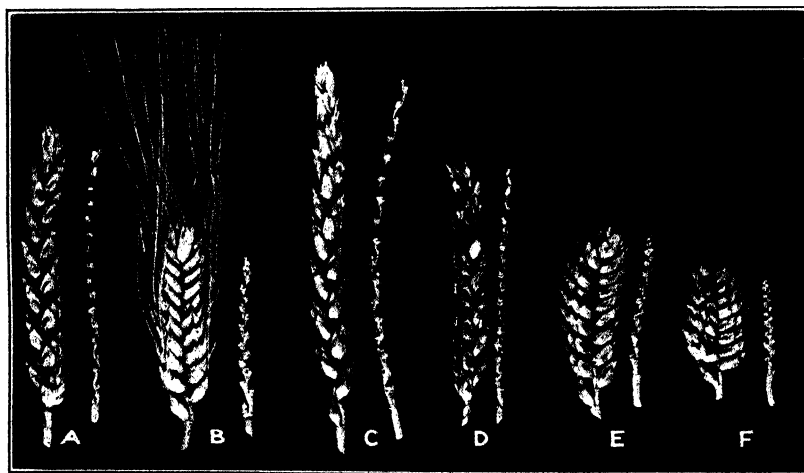


FIGURE 8.—Spikes and rachis from parents and progeny of the Federation \times III C-18 cross: A, Federation parent; B, III C-18 parent; C and D, spikes and rachis from the homozygous-lax F_3 progenies showing range of spike density; E and F, spikes and rachis from the homozygous-dense F_3 progenies showing range of spike density. C and D are from the most lax true-breeding F_3 progeny and E and F from the most dense

The F_1 spike density was 2.2 mm. per rachis internode. The heterozygous F_3 progenies came from F_2 plants which ranged from 1.6 to 2.8 mm. per rachis internode. Frequency curves of the F_2 parent plants which produced the three F_3 spike-density groups are shown in Figure 9.

There were 81 homozygous dense, 170 heterozygous, and 103 homozygous lax progenies. These numbers suggest a 1:2:1 ratio although $P=0.1942$, which is only a fair fit. The goodness of fit is given in Table 9.

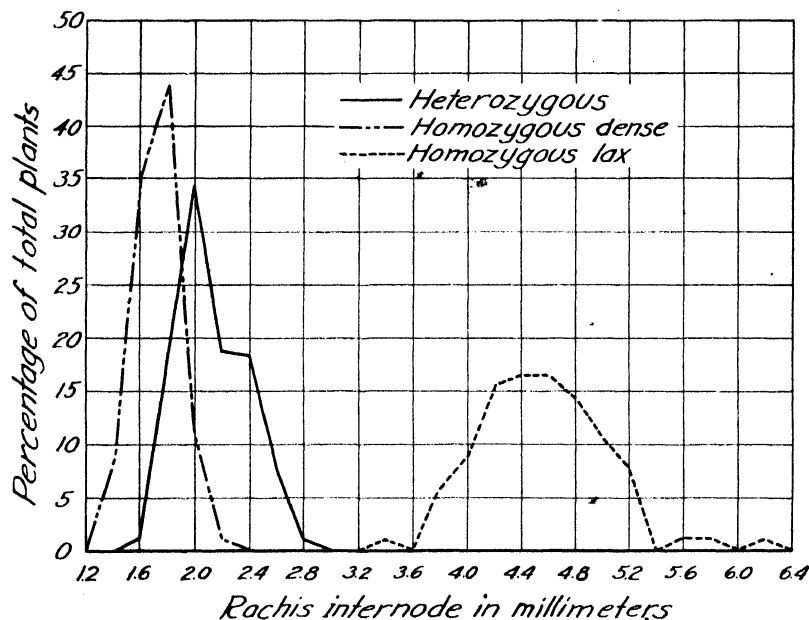
There is probably one major factor difference for spike density, and there may be some minor factors which modify this inheritance. This would seem to be indicated by the transgressive segregation which fails to recover the III C-18 parental head density (except in two cases at most, and not certainly in those) and by the rather wide range in the progenies of both homozygous groups.

TABLE 9.—Goodness of fit of three groups of F_3 progenies for spike density compared with a 1:2:1 ratio

[Grown in 1927 at Logan, Utah]

Progeny group	Calculated value (C)	Observed value (O)	O - C	(O - C) ²	$\frac{(O - C)^2}{C}$
Homozygous dense.....	88.5	81	-7.5	56.25	0.6356
Heterozygous.....	177.0	170	-7.0	49.00	.2768
Homozygous lax.....	88.5	103	+14.5	210.25	2.3757

$$\chi^2 = 3.3288. \quad P = 0.1942.$$

FIGURE 9.—Spike-density-distribution curves of F_3 plants which segregated in F_3 for the three major spike-density groups; the figure shows the spike-density curves of the F_3 progenies derived from these F_2 plants

CORRELATION OF CHARACTERS

AWN-CLASS AND SPIKE-DENSITY FACTORS PROBABLY NOT LINKED

Stewart (9) in a cross of Kanred \times Sevier obtained high correlations between awn-length and spike-density characters. In a cross of Federation \times Sevier (8) he obtained consistent correlation ratios (η) when spike density and awn classes were correlated. These results led to the belief that there was linkage between spike-density and awn-class factors.

In studying the present cross (Federation \times III C-18) with respect to linkage for spike-density and awn-class factors, there are two points which should be borne in mind: (1) Awn class as separated by eye classification is a distinct and separate character from awn length. There might be a high correlation between awn length of

fully awned progenies and another plant character, while the correlation between awn classes and the same plant character could be low or even essentially zero. Different awn-class groups may have awn length correlated with a given plant character in quite different degrees, as will be seen later in this discussion. (2) The awn-class inheritance in the Federation \times III C-18 cross is different from that of Federation \times Sevier cross, as has been already shown. Inheritance in the former showed independent segregation on the basis of a 2-factor difference, while that of the latter showed linkage.

TABLE 10.—Goodness of fit of 27 awn-class and spike-density genotype classes of F_3 progenies when compared with the theoretical ratio of independent segregation with 3-factor differences^a

[Grown in 1927 at Logan, Utah]

Class No.	Genotype	Calculated value (C)	Observed value (O)	O - C	(O - C) ²	$\frac{(O - C)^2}{C}$
1	AA BB LL	5.53	6	+0.47	0.2209	0.0399
2	AA BB Ll	11.06	9	-2.06	4.2436	.3837
3	AA Bb LL	11.06	8	-3.06	9.3636	.8466
4	Aa BB LL	11.06	12	+ .94	.8836	.0799
5	AA Bb Ll	22.12	26	+3.88	15.0544	.6906
6	Aa BB Ll	22.12	23	+ .88	.7744	.0350
7	AA Bb LL	22.12	24	+1.88	3.5344	.1598
8	Aa Bb Ll	44.24	38	-6.24	38.9376	.8801
9	AA BB ll	5.53	8	+2.47	6.1009	1.1032
10	AA Bb ll	11.06	4	-7.06	49.8436	4.5067
11	Aa BB ll	11.06	8	-3.06	9.3636	.8466
12	Aa Bb ll	22.12	19	-3.12	9.7344	.4401
13	AA bb LL	5.53	10	+4.47	19.9809	3.6132
14	AA bb Ll	11.06	8	-3.06	9.3636	.8466
15	Aa bb LL	11.06	15	+3.94	15.5236	1.4036
16	Aa bb Ll	22.12	19	-3.12	9.7344	.4401
17	aa BB LL	5.53	5	- .53	.2809	.0508
18	aa BB Ll	11.06	13	+1.94	3.7636	.3403
19	aa Bb LL	11.06	16	+4.94	24.4036	2.2065
20	aa Bb Ll	22.12	27	+4.88	23.8144	1.0766
21	AA bb ll	5.53	6	+ .47	.2209	.0399
22	Aa bb ll	11.06	12	+ .94	.8836	.0799
23	aa BB ll	5.53	6	+ .47	.2209	.0399
24	aa Bb ll	11.06	7	-4.06	16.4836	1.4904
25	aa bb LL	5.53	7	+1.47	2.1609	.3908
26	aa bb Ll	11.06	8	-3.06	9.3636	.8466
27	aa bb ll	5.53	10	+4.47	19.9809	3.6132
Total		353.92	354			

$\chi^2 = 26.4806$. $P = 0.4373$.

^a L = lax spike and l = dense spike; A and B = two factors for awns; AABB = fully awned condition.

In order to determine whether there was linkage between the awn-class and spike-density factors in the cross under study, the F_3 progenies were arranged into 27 genotype classes which would occur in independently segregating material where 3-factor differences are involved. Table 10 shows the genetic constitution of each of the 27 classes and also the goodness of fit when compared to the theoretical numbers which would be expected in a cross involving a 3-factor difference with independent segregation. The factor for lax spike is designated by L and its allelomorph dense spike by l. The awn classes have the same designation as before given.

For the 27 classes, $\chi^2 = 26.4806$ and $P = 0.4373$. This is a good fit, and indicates that there is at least no strong linkage between spike-density and awn-class characters in the Federation \times III C-18 cross.

PARENTS

Correlations were calculated for the Federation and the adjacent III C-18 parental rows. The correlation coefficients, their probable errors, and the $r/P.E.$ are given in Table 11.

TABLE 11.—Correlation coefficients (r), their respective probable errors ($P.E.$), and $r/P.E.$ for the pairs of parent rows when various plant characters were correlated

[Grown in 1927, at Logan, Utah]

Plant character	$r \pm P.E.$	$r/P.E.$
Culm length.....	$+0.5082 \pm 0.0318$	16.0
Spike density.....	$+0.0865 \pm .110$.8
Number of culms.....	$-.4770 \pm .0857$	5.6
Awn length.....	$+0.0093 \pm .1377$.1
Neck thickness.....	$+0.1240 \pm .1092$	1.1

The high positive correlation coefficient (r) obtained when the adjacent parental rows were correlated for culm length probably indicates that the soil heterogeneity influenced this character in a marked degree. The large minus correlation coefficient obtained when number of culms to the plant was considered probably indicates that a degree of competition existed between the two adjacent parental rows. Spike density ($r = +0.0865 \pm 0.110$), awn length ($r = +0.0093 \pm 0.1377$), and neck thickness ($r = +0.1240 \pm 0.1092$) seem to be more stable characters which are less influenced by environmental factors, as is shown by their low correlation coefficients.

Correlation coefficients were calculated for two pairs of plant characters studied for each parent. These constants with their probable errors are given in Table 12.

TABLE 12.—Correlation coefficients (r), their respective probable errors ($P.E.$), and $r/P.E.$ for two pairs of plant characters on each parent

[Grown in 1927 at Logan, Utah]

Parent and plant character	$r - P.E.$	$r/P.E.$
Federation:		
Culm length \times spike density.....	-0.0118 ± 0.1093	0.1
Spike density \times neck thickness.....	$+0.6373 \pm .0658$	9.7
III C-18:		
Culm length \times spike density.....	$-.2436 \pm .1043$	2.3
Spike density \times neck thickness.....	$+0.1877 \pm .1070$	1.8

Culm length \times spike density in the Federation parent rows gave a correlation coefficient which is only one-tenth of its probable error ($r = -0.0118 \pm 0.1093$). These same two characters in the III C-18 give a correlation coefficient which is of little significance ($r = -0.2436 \pm 0.1043$).

In Federation, the correlation coefficient between spike density \times neck thickness was large; that is, it was 9.7 times its probable error ($r = +0.6373 \pm 0.0658$). These same characters in the III C-18 gave a low correlation coefficient, which was only 1.8 times its probable error ($r = +0.1877 \pm 0.1070$). It seems from these data that spike density and neck thickness are distinctly correlated in the Federation parent. A laxer head tends to go with a thicker neck.

CORRELATION STUDIES WITH THE F_3 PROGENIES

Ten pairs of combinations of the five plant characters were possible. The correlation coefficients (r), the correlation ratios (η), and Blakeman's tests were calculated for all of the 10 pairs. These constants with their probable errors and $\frac{r}{P.E.}$ and $\frac{\eta}{P.E.}$ are given in Table 13.

TABLE 13.—Correlation coefficients (r), correlation ratios (η), their respective probable errors ($P. E.$), and Blakeman's test for linearity for various pairs of plant characters for 354 F_3 progenies

(Grown in 1927 at Logan, Utah)

Characters correlated	$r \pm P. E.$	$r/P. E.$	$\eta \pm P. E.$	$\frac{\eta}{P. E.}$	Blakeman's test
Culm length \times spike density.....	$+0.5198 \pm 0.0262$	19.8	0.5344 ± 0.0256	20.9	2.3740
Culm length \times awn length.....	$+ .0584 \pm .0417$	1.4	$.2993 \pm .0381$	7.9	3.5096
Culm length \times neck thickness.....	$+ .1974 \pm .0345$	5.7	$.2538 \pm .0335$	7.6	2.2263
Culm length \times number of culms.....	$+ .0521 \pm .0357$	1.5	$.1824 \pm .0347$	5.3	2.4427
Spike density \times awn length.....	$- .1112 \pm .0413$	2.7	$.3496 \pm .0367$	9.5	3.9641
Spike density \times neck thickness.....	$+ .4490 \pm .0286$	15.7	$.5711 \pm .0241$	23.7	4.9302
Spike density \times number of culms.....	$+ .0967 \pm .0355$	2.7	$.1833 \pm .0346$	5.3	2.1760
Awn length \times neck thickness.....	$- .2874 \pm .0384$	7.5	$.4068 \pm .0349$	11.7	3.4438
Awn length \times number of culms.....	$+ .0343 \pm .0418$.8	$.3107 \pm .0378$	8.2	3.6926
Neck thickness \times number of culms.....	$+ .0265 \pm .0358$.7	$.1755 \pm .0347$	5.1	2.4232

CULM LENGTH \times SPIKE DENSITY

The correlation coefficient for culm length \times spike density is large ($r = +0.5198 \pm 0.0262$). The constant is 19.8 times its probable error. The correlation ratio (η) is only slightly larger than r and Blakeman's test for linearity is only 2.3740, which indicates that the correlation is essentially linear in its nature.

Since this pair of characters when correlated in each of the two parents (Table 12) showed no significant constants, it was thought advisable to study the F_3 progenies more completely to determine, if possible, the nature of the relationship existing. In order to do this, the F_3 progenies were divided into their three spike-density groups—(1) homozygous dense, (2) heterozygous, and (3) homozygous lax. Correlations for culm length \times spike density were then calculated for each of the three groups independently. The correlation coefficients (r), their probable errors ($P. E.$), and $\frac{r}{P.E.}$ are given in Table 14.

TABLE 14.—Correlation coefficients (r) and probable errors ($P. E.$) for culm length \times spike density in each of the three spike-density groups of the F_3 progenies

(Grown in 1927 at Logan, Utah)

Spike-density group	$r \pm P. E.$	$r/P. E.$
Homozygous dense.....	$+0.2454 \pm 0.0709$	3.5
Heterozygous.....	$+ .1193 \pm .0509$	2.3
Homozygous lax.....	$- .1612 \pm .0648$	2.5

Two of the groups, homozygous dense ($r = +0.2454 \pm 0.0709$) and heterozygous ($r = +0.1193 - 0.0509$) show comparatively small plus correlation coefficients, and the third group, homozygous lax, shows a small minus correlation coefficient ($r = -0.1612 \pm 0.0648$). The largest constant of the three is only 3.5 times its probable error. These constants indicate that the large correlation coefficient ($r = +0.5198 \pm 0.0262$) obtained when the entire 354 F_3 progenies were included is probably between groups (homozygous dense, heterozygous, and homozygous lax) rather than within the groups.

The spike-density factors seem strongly to influence culm length. This is somewhat in keeping with Boshnakian's findings (2). This is difficult to explain since the parental forms bring the characters into the cross in the opposite combination from that shown in the progenies. The lax-spiked parent (Federation) is the short parent and the dense-spiked parent (III C-18) is the tall parent. It has already been noted that spike-density inheritance is rather complicated. It may be that culm-length inheritance is also complicated.

CULM LENGTH \times AWN LENGTH

The correlation coefficient between culm length and awn length is small ($+0.0584 \pm 0.0417$), and is only 1.4 times its probable error. So far as r can measure, no correlation exists between these characters. The correlation ratio $= 0.2993 \pm 0.0381$ and is 7.9 times its probable error. This constant, together with the rather high figure determined for Blakeman's test for linearity (B. T. = 3.5096), suggests the possibility of a correlation which r is unable to measure.

TABLE 15.—Correlation coefficients (r) and their respective probable errors for each of the four homozygous awn-class groups of F_3 progenies

[Grown in 1927 at Logan, Utah]

Characters correlated	$r \pm P. E.$	$r/P. E.$	Characters correlated	$r \pm P. E.$	$r/P. E.$
Awn class 4:			Awn class 3—Contd.		
Culm length \times spike density	$+0.4857 \pm 0.1074$	4.5	Awn length \times neck thickness	$+0.1462 \pm 0.1348$	1.1
Culm length \times awn length	$+0.7226 \pm 0.0672$	10.8	Awn class 2:		
Culm length \times neck thickness	$+0.4737 \pm 0.109$	4.3	Culm length \times spike density	$+0.2750 \pm 0.1300$	2.1
Spike density \times awn length	$+0.7857 \pm 0.0538$	14.6	Culm length \times awn length	-0.0075 ± 0.1406	0.05
Spike density \times neck thickness	$+0.5867 \pm 0.0922$	6.4	Culm length \times neck thickness	-0.4676 ± 0.1099	4.3
Awn length \times neck thickness	$+0.5451 \pm 0.0989$	5.5	Spike density \times awn length	$+0.0382 \pm 0.1404$	3.6
Awn class 3:			Spike density \times neck thickness	$+0.4492 \pm 0.1122$	4.0
Culm length \times spike density	$+0.5584 \pm 0.0948$	5.9	Awn length \times neck thickness	$+0.1709 \pm 0.1365$	1.3
Culm length \times awn length	$+0.0232 \pm 0.1376$	0.2	Awn class 1:		
Culm length \times neck thickness	-0.0315 ± 0.1375	0.2	Culm length \times spike density	$+0.7991 \pm 0.0488$	16.4
Spike density \times awn length	$+0.2162 \pm 0.1313$	1.6	Culm length \times neck thickness	$+0.6777 \pm 0.0729$	9.3
Spike density \times neck thickness	$+0.5697 \pm 0.0930$	6.1	Spike density \times awn length	$+0.5924 \pm 0.0876$	6.8
			Spike density \times neck thickness		

Table 15 gives correlations between various plant characters when the progenies are grouped into the true-breeding awn-class groups. The awn 4 group gives a high correlation coefficient ($r = +0.7226 \pm 0.0672$) between culm length and awn length, which is 10.8 times its

probable error. Awn 3 ($r = +0.0232 \pm 0.1376$) and awn 2 groups ($r = -0.0075 \pm 0.1406$) give very low correlation coefficients, which in both cases are considerably less than their probable errors.

The correlation which η suggested seems to be located in fully awned progenies but not in the progenies of the other awn classes.

CULM LENGTH AND NECK THICKNESS

The correlation coefficient obtained for these two factors was $+0.1974 \pm 0.0345$. The constant is 5.7 times its probable error. The correlation ratio obtained ($\eta = 0.2538 \pm 0.0335$) is 7.6 times its probable error. Blakeman's test was 2.2263.

The awn 4 group has a fairly high plus correlation coefficient ($r = +0.4737 \pm 0.109$ and $\frac{r}{P.E.} = 4.3$). The awn 3 group has a correlation coefficient lower than its probable error ($r = -0.0315 \pm 0.1375$).

The awn 2 group has a rather high minus correlation coefficient ($r = -0.4676 \pm 0.1099$), which is 4.3 times its probable error. Awn 1 group has a high plus correlation coefficient ($r = +0.6777 \pm 0.0729$). It is 9.3 times its probable error.

Two of the four awn groups, then, show significant plus correlations. One of the other two groups shows a high minus correlation coefficient and the other a small minus correlation. It is apparent that since the correlations in two of the four are rather high plus and two are minus they would tend so to counterbalance each other when r was calculated for the entire family that a small r would be obtained.

CULM LENGTH \times NUMBER OF CULMS

The correlation coefficient obtained for these characters is very small ($r = +0.0521 \pm 0.0357$), and is only 1.5 times its probable error. The correlation ratio obtained ($\eta = 0.1824 \pm 0.0347$) is probably not very significant when viewed in connection with the small Blakeman's test obtained (B. T. = 2.4427).

SPIKE DENSITY \times AWN LENGTH

For these characters the correlation coefficient obtained ($r = -0.1112 \pm 0.0413$) is small, and is only 2.7 times its probable error.

The correlation ratio ($\eta = 0.3496 \pm 0.0367$) is fairly good sized, and is 9.5 times its probable error. Blakeman's test (B. T. = 3.9641) is also fairly high. These two constants indicate a correlation which r fails to measure. The correlation coefficients between spike density and awn length in the four awn groups are:

Awn group 4, $r = +0.7857 \pm 0.0538$ and $\frac{r}{P.E.} = 14.6$

Awn group 3, $r = +0.2162 \pm 0.1313$ and $\frac{r}{P.E.} = 1.6$

Awn group 2, $r = +0.0382 \pm 0.1404$ and $\frac{r}{P.E.} = 0.3$

The correlation indicated by η seems to be located again, as was the correlation of culm length \times awn length, in the fully awned group, and to be lacking in the other awn groups studied. It was in fully

awned progenies that strong linkage was obtained between spike density and awn length in the Sevier \times Kanred cross (6).

The value of η in indicating correlations which r does not measure is again brought out, rather clearly, in this instance.

SPIKE DENSITY \times NECK THICKNESS

The correlation coefficient for these two characters is perhaps high enough to be significant ($r = +0.4490 \pm 0.0286$ and $\frac{r}{P. E.} = 15.7$).

A still higher correlation ratio ($\eta = 0.5711 \pm 0.0241$ and $\frac{\eta}{P. E.} = 23.7$) and a high Blakeman's test (B. T. = 4.9302) both suggest that there may be considerable correlation which r fails to measure.

For these same two characters Federation gave a correlation coefficient (Table 12) of $+0.6373 \pm 0.0658$ and $\frac{r}{P. E.} = 9.69$. For III C-18, r was $+0.1877 \pm 0.1070$ and $\frac{r}{P. E.} = 1.75$. The laxer of the two parents gave a good plus correlation. The denser parent gave a plus correlation which is probably too small to be significant.

In order to study these characters more fully, correlation coefficients were calculated for them in each of the three spike-density groups (homozygous dense, heterozygous, and homozygous lax). The coefficients of correlation with their probable errors are given in Table 16.

TABLE 16.—Correlation coefficients (r) and probable errors ($P. E.$) for spike density \times neck thickness in each of the three spike-density groups of F_3 progenies

[Grown in 1927 at Logan, Utah]

Spike-density group	$r \pm P. E.$	$r/P. E.$
Homozygous dense.....	$+0.3917 \pm 0.0638$	6.1
Heterozygous.....	$+0.2612 \pm 0.0481$	5.4
Homozygous lax.....	$+0.6915 \pm 0.0347$	19.2

The correlation coefficient for homozygous dense ($r = +0.3917 \pm 0.0638$) is 6.1 times its probable error. For the heterozygous ($r = +0.2612 \pm 0.0481$) the constant is 5.4 times its probable error. For homozygous lax ($r = 0.6915 \pm 0.0347$) the constant is 19.2 times its probable error. While these coefficients are all plus and all high enough to be significant, there is a wide difference between the lowest and the highest. For indicating such a spotted or curvilinear condition in a population, η seems to be valuable.

Plus correlation coefficients were obtained, then, for the entire family, for both parents, and for each of the three spike-density groups. All were high enough to be significant except the III C-18 constant. (Table 12.) Spike density and neck thickness are, then, positively correlated.

All the data indicate that the laxer the spike the thicker the neck, and the denser the spike the thinner the neck. Since this relationship occurs consistently with the entire family, with the parents

(although to a much less extent with III C-18, the denser parent), and with the four homozygous awn groups studied, the suggestion is that it may be a physiological relationship.

SPIKE DENSITY AND NUMBER OF CULMS

These characters gave a small correlation coefficient ($r = +0.0967 \pm 0.0355$ and $\frac{r}{P.E.} = 2.9$). The correlation ratio was somewhat higher but not significantly so ($\eta = 0.1833 \pm 0.0346$ and $\frac{r}{P.E.} = 5.3$). Blakeman's test for linearity was 2.1760.

AWN LENGTH \times NECK THICKNESS

The correlation coefficient for these characters is -0.2874 ± 0.0384 , which is 7.5 times its probable error. The correlation ratio is 0.4068 ± 0.0349 . It is 11.7 times its probable error. Blakeman's test for linearity is 3.4438, and suggests a stronger correlation than r measured.

A study of the 4 awn group throws some light on this (Table 15). Awn group 4 has a good correlation coefficient ($r = +0.5451 \pm 0.0989$).

The correlation coefficient for the awn 3 group is $+0.1462 \pm 0.1348$, and for the awn 2 group $+0.1709 \pm 0.1365$. In this case as well as in others previously discussed, the correlations are much stronger in awn group 4 than in the other awn groups.

AWN LENGTH \times NUMBER OF CULMS

The correlation coefficient for these characters is $+0.0343 \pm 0.0418$. The correlation ratio is 0.3107 ± 0.0378 and is 8.2 times its probable error, and Blakeman's test is 3.6926. These latter figures suggest that there might be a correlation not measured by r . If such a correlation existed it was not found.

NECK THICKNESS \times NUMBER OF CULMS

This correlation coefficient is $+0.0265 \pm 0.0358$. It is smaller than its probable error. The correlation ratio ($\eta = 0.1755 \pm 0.0347$) is 5.1 times its probable error. Blakeman's test is 2.4232.

SUMMARY

III C-18 (a hybrid strain from a Sevier \times Dicklow cross) and Federation were crossed. A vigorous F_1 family was chosen to continue the F_2 generation. Grain from each F_2 plant was used to seed one F_3 progeny row. The rows were spaced 1 foot apart and the kernels in the row were spaced about 3 inches apart. Paired plantings of the two parents were made in the same manner and at the same time after each 10 progeny rows.

Each F_3 progeny row was considered to represent the genetic constitution of its F_2 parent plant. There were 354 F_3 progenies. Data were collected for the various plant characters studied by observation or by measurement, as follows: Color of glume, awn class, culm

length, spike density, awn length, neck thickness, and number of culms.

Color of glume was found to be inherited on the basis of a 1-factor difference. The numbers obtained were 82 bronze, 176 heterozygous, and 96 whites. These numbers closely approach a 1:2:1 ratio. $P=0.5782$.

Awn classes were found to be inherited on the basis of a 2-factor difference with independent segregation. The numbers expected on this hypothesis very closely fit the observed facts. $P=0.9270$ and indicates an extremely good fit.

Culm length of the F_3 progenies was correlated with their F_2 parents to the extent of $+0.4316 \pm 0.0291$, which indicates that segregation occurred. The culm-length range in the parental rows was covered by the F_3 progenies.

Spike-density inheritance was peculiar in its nature. Three spike-density groups were found in the F_3 progeny—(1) homozygous dense, (2) heterozygous, and (3) homozygous lax with the respective numbers for each group of 81, 170, and 103. These numbers suggest a 1:2:1 ratio, with the goodness of fit as indicated by $P=0.1942$. The homozygous-dense progenies transgress the range of the dense parent in mean spike density. The dense parent III C-18 is possibly not recovered in a single progeny, and certainly not in more than two. The homozygous-dense progenies with these two possible exceptions are all more dense than the most dense of the III C-18. The group of heterozygous progenies fall between the two homozygous groups of progenies with no overlapping. (Fig. 7.) The range of densities of the heterozygous progenies cover that of the denser parent entirely. There is probably a 1-factor difference with perhaps some minor factors involved in the spike-density inheritance of this cross.

The awn-length range of the parental rows was recovered in the F_3 progenies. Homozygous awn group 1 was essentially the same as Federation in awn length. Homozygous awn 4 group recovered the greatest parental awn length. The other awn classes were graduated in length in rather regular order between awns 1 and awns 4. Standard deviations and coefficients of variability were distinctly higher for the segregating awn class genotypes than for the homozygous awn classes.

Neck thickness was essentially the same for the two groups of parent rows. The range in the progenies was small.

The number of culms was essentially the same in both parents and F_3 progenies.

Awn-class and spike-density characters were studied together to determine whether or not linkage existed. The 354 F_3 progenies were arranged into 27 genotype groups, which might be expected on the hypothesis of independent segregation with a 3-factor difference (two for awn classes and one for spike density). Their numbers approached the expected numbers. When goodness of fit was calculated, $P=0.4373$. This indicates that there is probably no linkage between spike-density and awn-class factors in this cross.

Correlation coefficients (r) and correlation ratio (η) and Blake-man's tests were calculated for various combinations of the following plant characters: Culm length, spike density, awn length, neck thickness, and number of culms.

Culm length seems not to be correlated with spike density in either of the parents, or in either of the three spike-density groups—homozygous dense, heterozygous, and homozygous lax. However, when the correlation coefficient was calculated for the entire family of the F_3 progenies there was a significant correlation, that is, the factors for the spike-density classes seem to have a distinct influence on culm length.

Culm length and awn length are distinctly correlated in the homozygous fully awned progenies. The other homozygous awn-class groups show no correlation. The entire family shows no correlation, yet $\eta(\eta = 0.2993 \pm 0.0381$ and $P \cdot \frac{\eta}{E} = 9.2$) and Blakeman's test ($B. T. = 3.5096$) indicated the presence of a correlation, which was found in the fully awned group.

Spike density and awn length also seem to be correlated in the homozygous awn 4 group but not elsewhere. This correlation had also been indicated by the correlation ratio (η).

Spike density and neck thickness were found to be correlated in the Federation parent, in the four homozygous awn groups studied, and in the entire family.

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COMPOSITION OF COMMERCIAL ACID LEAD ARSENATE AND ITS RELATION TO ARSENICAL INJURY¹

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INTRODUCTION

Since acid lead arsenate was first used as an insecticide in 1892, its manufacture has become relatively well stabilized and a fairly uniform product is assured. Analyses of a considerable number of samples over a period of several years, however, show that more or less variation occurs in the quantities of arsenious oxide, total arsenic, and water-soluble arsenic present.

The total proportion of arsenic theoretically present in pure acid lead arsenate, expressed as arsenic pentoxide, is 33.1 per cent. Actually it was found to range from 28.0 to 32.8 per cent, thus indicating that commercial acid lead arsenate contains more or less basic lead arsenate. The more of the basic form present the safer the material is on foliage and the less toxic it is to insects (3, p. 50; 6; 9; 10).²

Although arsenious oxide should theoretically be absent, it was found to be present in quantities ranging from 0.16 to 1.40 per cent. As far as the writer is aware, the relation of the presence of this material to foliage injury has not been previously investigated.

The content of the water-soluble arsenic ranged from 0.04 to 5.93 per cent as arsenic pentoxide. The maximum guaranteed ranges from 0.38 to 1.15 per cent, although as a rule manufacturers guarantee their product to contain not more than 0.75 per cent. Little information has been available as to the effect of these quantities of water-soluble arsenic and the necessary minimum to avoid foliage injury. The quantity of soluble arsenic pentoxide present is regarded by some as of little importance so long as it is below the 0.75 per cent maximum set for lead arsenate paste by the insecticide act of 1910, which has been rather generally adopted by manufacturers for the powdered form now used. Others consider it of great importance, extravagant claims often being made by manufacturers and salesmen for a particular brand of lead arsenate as noninjurious to foliage because of its low soluble-arsenic content.

A series of experiments was conducted to determine the effect of these variations in composition upon foliage injury. All spraying tests were conducted upon peach trees, which are perhaps the most sensitive to arsenical injury of all the plants to which acid lead arsenate is extensively applied. These tests were carried on from 1926 to 1928 in central Georgia.

¹ Received for publication Feb. 26, 1929; issued September, 1929.

² Reference is made by number (italic) to "Literature cited," p. 400.

TOXIC CONCENTRATIONS OF ARSENIOS AND ARSENIC ACIDS

To determine the minimum concentration of soluble arsenic toxic to peach foliage, solutions of arsenious and arsenic acids were made up in concentrations ranging from 0.0001 to 3.0 per cent expressed as arsenic pentoxide.³ These were applied to peach trees with a hand sprayer until the spray material dripped from the foliage, and the results were noted daily. A summary of the results of these tests is given in Table 1.

TABLE 1.—*Effect of arsenious and arsenic acids, in various concentrations, upon peach foliage*

Concentration of acid used (as As ₂ O ₅)	H-ion concentrations *		Time from spraying to first injury		Final effect on foliage	
	As ₂ O ₃	As ₂ O ₅	As ₂ O ₃	As ₂ O ₅	As ₂ O ₃	As ₂ O ₅
Per cent	pH	pH	Days	Days		
0.0001	6.4	6.3			No injury.	No injury.
.0005	6.4	6.1			do.	Do.
.0010	6.4	5.9			do.	Do.
.0015	6.35	5.5	8	8	Light leaf burning.	Light leaf burning.
.0020	6.35	5.1	8	8	do.	Do.
.0025	6.35	4.6	5	5	Moderate leaf burning.	Moderate leaf burning.
.0050	6.35	3.5	5	5	Severe leaf burning.	Severe leaf burning.
.0075	6.3	3.2	5	5	Severe leaf burning, light twig cankering, moderate defoliation.	Severe leaf burning, light twig cankering, light defoliation.
.0100	6.3	2.6	5	3	do.	Do.
.0200	6.3	2.5	5	3	Severe defoliation, light cankering.	Severe defoliation, severe cankering.
.0300	6.3	2.5	5	2	Severe defoliation, severe cankering.	Complete defoliation, very severe cankering.
.0400	6.25	2.45	5	2	Complete defoliation, severe cankering.	All twigs killed.
.0500	6.2	2.4	5	2	do.	Do.
.1000	5.4	2.2	3	2	All twigs killed.	Do.
.2000	5.3	2.1	3	1	do.	Do.
.3000	5.2	2.0	3	1	do.	Do.
.4000	5.1	1.9	2	1	do.	Do.
.5000	5.0	1.9	1	1	do.	Do.
.6000	4.9	1.85	1	1	do.	Do.
.7000	4.85	1.8	1	1	do.	Do.
.8000	4.8	1.75	1	1	do.	Do.
.9000	4.7	1.75	1	1	do.	Do.
1.0000	4.6	1.6	1	1	do.	Do.
1.5000	3.8	1.5	1	1	do.	Do.
2.0000	3.6	1.5	1	1	do.	Do.
2.5000	3.4	1.5	1	1	do.	Do.
3.0000	3.3	1.5	1	1	do.	Do.

* Hydrogen-ion concentration of water used, pH 6.5.

DISCUSSION OF RESULTS

At low concentrations (below 0.01 per cent as arsenic pentoxide) the arsenious and arsenic acids were of equal toxicity when compared upon the basis of metallic arsenic content. Upon the same basis, at high concentrations arsenic acid was the more toxic, possibly because of its greater penetrative power due to its higher acidity. This difference in toxicity occurs at acidities of arsenic acid of pH 2.6 and higher.

No difference in toxicity between arsenious and arsenic acids was apparent at the concentrations at which either is present as soluble arsenic in lead arsenate.

³ The percentages are based upon grams of solute per hundred grams of solution.

The minimum concentration toxic to peach leaves contained the equivalent of 0.0015 per cent arsenic pentoxide.

TESTS OF ARSENIC AS A CUMULATIVE POISON

As the arsenic upon a leaf can be in solution only when water is present, its action is more or less intermittent. To determine the minimum toxic concentration under these conditions and to ascertain whether arsenic might act as a cumulative poison, the experiments reported in Table 2 were performed. Concentrations under the toxic dosage of 0.0015 per cent arsenic pentoxide were applied successively to the same foliage and the results noted. A record of the rainfall during this period is given in Table 3. On June 30 it began to rain nine hours after the sprays had been applied, and on July 5, five hours afterwards. On July 13 and 16 spraying was begun approximately one hour after it had ceased raining.

TABLE 2.—*The cumulative effect of repeated applications of arsenic acid, in various concentrations, to peach foliage from June 29 to July 22, 1928*

Plot No.	Percent- age of As ₂ O ₅ used	Dates sprayed	Effect on foliage
1	0.0003	June 29	No injury.
2	.0003	June 29, 30	Do.
3	.0003	June 29, 30, July 2	Do.
4	.0003	June 29, 30, July 2, 3	Very light burning of leaves.
5	.0003	June 29, 30, July 2, 3, 5	Moderate burning of leaves.
6	.0015	June 29	Light burning of leaves.
7	.0003	June 29, 30	Do.
	.0009	July 13	
8	.0009	do	No injury.
9	.0008	July 16, 19	Moderate burning of leaves.
10	.0008	July 16, 22	Do.
11	.0008	July 16	No injury.

TABLE 3.—*Rainfall during the period of the tests of Table 2, June 29 to July 22, 1928*

Date	Rainfall	Date	Rainfall
	<i>Inches</i>		<i>Inches</i>
June 30	Trace.	July 12	1.13
July 5	0.68	July 13	2.80
July 6	.33	July 16	2.00
July 9	.43	July 17	.31
July 10	.04	July 18	Trace.
July 11	.50	July 21	.40

DISCUSSION OF RESULTS

The results on plots 1 to 6 showed that slight injury could occur with a minimum concentration equivalent to 0.0012 per cent arsenic pentoxide, although a concentration equivalent to 0.0015 per cent was necessary before sufficient injury occurred to be of importance. It was also apparent that slightly greater injury occurred when this quantity of soluble arsenic was applied in five sprays of 0.0003 per cent each than when it was applied in one spray of 0.0015 per cent arsenic pentoxide. This is probably due to the greater quantity of water furnished and to the smaller loss of poison in the former case. Plots

7 to 11 offer evidence that arsenic is a cumulative poison within the peach leaf, as sufficient rain intervened between applications to remove any arsenic left upon the surface of the leaf. In plot 7 sufficient arsenic remained within the leaves from two applications of 0.0003 per cent arsenic pentoxide to cause injury when a third application of 0.0009 per cent was made 13 days later, the injury being only slightly less severe than from one application of 0.0015 per cent. These results indicate that the disposal of this poison by the leaf is at least very slow and that arsenic may therefore be termed a cumulative poison.

SOLUBLE ARSENIC IN ACID LEAD ARSENATES

A summary is given in Table 4 of the toxic concentrations of arsenic acid and the equivalent percentages of soluble arsenic in powdered lead arsenate when used in the proportion of 1 pound to 50 gallons of water.

TABLE 4.—*Minimum concentrations of arsenic acid causing various types of injury to peach foliage*

Extent of injury	Minimum concentrations of soluble arsenic pentoxide causing injury specified	Equivalent to the percentage found in the spray when the lead arsenate (used in the proportion of 1 pound to 50 gallons of water) has a soluble arsenic content of—
	<i>Per cent</i>	<i>Per cent</i>
Trace of leaf burning.....	0.0012	0.50
Light leaf burning.....	.0015	.62
Moderate leaf burning.....	.0025	1.04
Severe leaf burning.....	.0050	2.08
Light defoliation.....	.0075	3.11
Complete defoliation.....	.0300	12.45
All twigs killed.....	.0500	20.75

TABLE 5.—*Soluble arsenic determinations in distilled water and in well water*

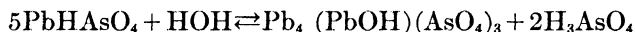
Soluble As_2O_3 present when digested at 32° C. for 24 hours in—

Sample No.	Distilled water		Well water used in spraying (pH 6.4)
	Unboiled (pH 5.7)	Boiled (pH 6.1)	
1.....	<i>Per cent</i> 0.092	<i>Per cent</i> 0.112	<i>Per cent</i> 0.505
2.....	.172	.221	.650

If it be assumed that all the injury from the use of acid lead arsenate is caused by the arsenic that becomes soluble in the spray tank, it would appear from Table 4 that a lead arsenate with soluble arsenic

in quantities less than 0.50 per cent as arsenic pentoxide would be perfectly safe upon peach foliage. This of course refers to the soluble arsenic formed in the water used in spraying. According to the official methods (1), the solubility is determined in boiled distilled water which gives much lower values, as shown in Table 5, owing to its greater acidity and lack of buffering power. Where more alkaline waters are used in making up the spray, even greater differences result. Granting the above assumption, therefore, a lead arsenate containing less than 0.11 per cent of soluble arsenic pentoxide according to the official methods would seem necessary to prevent injury.

This assumption, however, is untrue, as arsenic has been shown to be a cumulative poison, and the repeated absorption of much smaller quantities of soluble arsenic by the leaves resulted in injury. Therefore, if water from frequent rains or dew is available upon the leaf for the hydrolysis of the lead arsenate, injury would be expected to occur even when an arsenate of very low soluble-arsenic content was used. The only safe acid lead arsenate would be one which was not at all hydrolyzed by water. This apparently is impossible to obtain, as even the purest acid lead arsenate is slowly hydrolyzed by the action of water according to the following equation (7, p. 1918):



As the soluble arsenic acid thus formed is constantly being removed by passage through the leaf epidermis, the reaction continues to move to the right whenever moisture is available, and a concentration of arsenic sufficient to cause very severe burning or even defoliation often accumulates within the leaves.

Although it is apparently impossible to produce an acid lead arsenate with soluble-arsenic content so low that it will not burn the leaves, it is evident that one containing a high percentage of soluble arsenic is very dangerous to foliage.

FIELD-SPRAYING TESTS

To determine from a practical standpoint just how low the soluble arsenic should be for minimum injury, a series of lead arsenates with contents of soluble arsenic pentoxide ranging from 0.04 to 1.19 per cent were used upon peach trees during the summers of 1927 and 1928. These were applied with a double-acting hand pump at 160 pounds pressure. All were used in the proportion of 1 pound to 50 gallons of water and each tree was sprayed until the spray dripped from the leaves. All arsenates were analyzed by the official methods (1). These analyses and the results of the spraying tests are given in Table 6. High humidity and frequent rains throughout both of the experimental periods provided an abundance of moisture for the hydrolysis of the acid lead arsenate, conditions during 1928 being much the more favorable to burning in this respect.

DISCUSSION OF RESULTS

Perhaps the most important fact brought out by these experiments is that acid lead arsenate when used alone in the proportion of 1 pound to 50 gallons caused very severe injury even when the

soluble arsenic was as low as 0.04 per cent as arsenic pentoxide. Apparently the soluble arsenic can not be sufficiently reduced to prevent burning.

TABLE 6.—Summary of field experiments in spraying peach trees with lead arsenate solutions (1 pound to 50 gallons) containing varying percentages of soluble arsenic pentoxide, performed during 1927 and 1928

EXPERIMENTS OF 1927

Sample	Total As ₂ O ₃	Total As ₂ O ₃ (as As ₂ O ₅)	Water-soluble arsenic as As ₂ O ₃	Interval between application and—					Final extent of injury
				Initial injury	Moderate leaf burning	Initial defoliation	Severe defoliation	Complete defoliation	
	Per cent	Per cent	Per cent	Days	Days	Days	Days	Days	
1.-----	31.29	0.16	0.13	4	7	6	14	-----	Severe defoliation.
2.-----	29.89	.31	.17	4	7	5	14	-----	Do.
3.-----	32.26	.45	.40	4	7	5	8	-----	Very severe defoliation.
4.-----	32.21	.45	.63	4	7	5	8	-----	Do.
5.-----	31.28	1.14	.88	3	7	5	8	8	Complete defoliation.
6.-----	31.81	1.40	1.19	2	5	5	7	8	Do.

EXPERIMENTS OF 1928

A.-----	32.80	.25	.04	4	7	11	22	31	Complete defoliation, very light twig cankering.
B.-----	29.90	.40	.11	5	7	11	27	37	Do.
C.-----	32.46	.44	.12	4	7	11	18	27	Complete defoliation, light twig cankering.
D.-----	31.68	.44	.16	4	7	8	18	27	Do.
E.-----	29.50	.30	.22	4	6	8	18	27	Do.
F.-----	32.21	.45	.61	3	5	8	11	13	Complete defoliation, severe twig cankering.
G.-----	31.28	.45	.82	3	4	6	11	13	Complete defoliation, very severe twig cankering.

There was no appreciable difference in the injury produced by a lead arsenate containing 0.04 per cent and by those containing up to 0.22 per cent water-soluble arsenic pentoxide. When arsenates containing higher percentages of soluble arsenic were used, the injury took place more rapidly and the final injury was more severe.

WATER-SOLUBLE ARSENIC IN CHEMICALLY PURE AND IN COMMERCIAL ACID LEAD ARSENATES

Chemically pure acid lead arsenate was prepared by twice crystallizing from nitric acid according to Duvillier's method (8). This was washed thoroughly with acidified water (pH 4.0), dried at 110° C. to constant weight, and analyzed. It was found to contain 33.0 per cent arsenic pentoxide and 64.1 per cent lead oxide, thus proving it to be pure acid lead arsenate. After being digested with boiled distilled water (pH 6.4) for 24 hours at 32° according to the official methods (1), it was found that 0.04 per cent of its weight as arsenic pentoxide had dissolved in water.

Commercial acid lead arsenates are practically never as low as this in soluble arsenic, even after repeated washing. Numerous analyses were made to determine the causes of the comparatively large quantities of soluble arsenic, and typical examples of these results are given in Table 7. All analyses were made by official methods.

TABLE 7.—Soluble arsenic found in various samples of acid lead arsenate before and after washing

Sample No.	Total As ₂ O ₃	Total As ₂ O ₅	pH ^a	Alkaline material present	Soluble arsenic as As ₂ O ₅	
					Initial	After washing ^b
	Per cent	Per cent			Per cent	Per cent
1.....	0.450	32.21	5.4	None.....	0.001	0.063
2.....	.396	29.50	6.4	Excess lead oxide.....	.158	.132
3.....	.450	31.28	7.8	Calcium.....	.759	.139
4.....	1.400	32.40	5.4	None.....	1.190	.200
5 ^c130	33.00	4.8	do.....	.040	.040

^a 1 gm. powder to 10 c. c. distilled water; shaken, and pH determined after 10 minutes.

^b Washed four times with water acidified with nitric acid (pH 4.0).

^c Chemically pure lead arsenate prepared by the writer.

SOURCES OF SOLUBLE ARSENIC IN THE SAMPLES

The soluble arsenic in sample 1 (Table 7) was greatly reduced by washing. Since analyses failed to disclose the reason for the large quantity of soluble arsenic present, a sample was boiled for one hour in water made acid with nitric acid (pH 3.0), dried, and again analyzed. The soluble arsenic content was then found to be 0.10 per cent as arsenic pentoxide, indicating that in this case sufficient time for reaction during manufacture had not been given.

In sample 2 the soluble arsenic was not greatly reduced by washing, and the greater quantities were apparently due to more rapid hydrolysis. The speed of hydrolysis of acid lead arsenate increases with an increase in OH-ion concentration, and the pH value of this sample was found to be somewhat higher than that of pure lead arsenate. This is apparently due to the excess of lead oxide, which has a slightly alkaline reaction. A large excess of lead oxide is apparently to be avoided, although a slight excess is unavoidable under commercial conditions.

In sample 3 also the large quantity of soluble arsenic was due to more rapid hydrolysis caused by higher alkalinity. In this case calcium was found to be present, and upon inquiry it was found that hydrated lime had been added during manufacture to neutralize any free acids present. The addition of materials with an alkaline reaction should always be avoided where possible.

In sample 4 the large quantity of soluble arsenic was apparently due to the arsenic trioxide present. The arsenic trioxide used in manufacture had been incompletely oxidized, and a small quantity was present in a loosely bound form, probably as lead arsenite. The soluble-arsenic content has invariably been observed to be high when a large quantity of arsenic trioxide is present in lead arsenate.

In sample 5 the soluble arsenic was apparently formed by hydrolysis of pure acid lead arsenate. The quantity formed was low and was not changed by repeated washing with acidified water.

No samples were found in which the soluble arsenic was due to the use of an insufficient quantity of lead.

EFFECT OF LOW SOLUBILITY UPON TOXICITY TO INSECTS

As it has been shown that injury can be reduced, but not prevented, by the reduction in soluble arsenic pentoxide below 0.25 per cent,

and that it is possible to produce a lead arsenate as low as this or lower in soluble arsenic, there yet remains the question as to whether the toxicity to insects is lowered by this reduction in solubility.

The digestive juices within an insect's digestive tract which are available for reaction with lead arsenate have been found (2, p. 139; 4; 5) to have a slightly alkaline reaction (pH above 7.0). Tests conducted by the writer have shown that hydrolysis of lead arsenate takes place rapidly in solutions with pH values above 6.0. Under these conditions such small differences in initial soluble arsenic would be expected to have little or no influence upon toxicity.

Cook and McIndoo (3, p. 42), as the result of rather extensive toxicity tests upon various insects with a number of arsenates, came to the following conclusions: (1) No differences in mortality could be attributed to the usual differences in the water-soluble arsenic content found in insoluble arsenates; (2) toxicity seemed to be based upon the stability of a compound and upon how easily it could be broken down in the bodies of insects.

Apparently the initial soluble arsenic, within ordinary limits, has little or no effect upon toxicity, and it would seem safe to conclude that the soluble-arsenic content of acid lead arsenate could be reduced below 0.25 per cent as arsenic pentoxide without loss in toxicity to insects.

SUMMARY

At low concentrations of equivalent arsenic content, arsenious and arsenic acids are equally toxic to peach foliage. At higher concentrations arsenic acid is the more toxic.

Arsenic acts as a cumulative poison within peach leaves.

The minimum concentration of arsenic acid toxic to peach foliage contains the equivalent of 0.0012 per cent of arsenic pentoxide.

Acid lead arsenates containing less than 0.25 per cent of arsenic pentoxide in water-soluble form gave minimum foliage injury. Nothing of practical importance was gained by further reductions in soluble arsenic.

It is apparently impossible to reduce the soluble arsenic in acid lead arsenate sufficiently to prevent serious injury when used upon tender foliage. It is therefore evident that acid lead arsenate can not be safely used upon susceptible plants without the addition of some material to prevent burning.

The initial soluble arsenic, within ordinary limits, has little or no effect upon the toxicity of acid lead arsenate to insects.

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INFECTION OF FRUIT OF CITRUS BY PSEUDOMONAS CITRI¹

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INTRODUCTION

The present studies were made for the most part on citrus fruits grown in an isolated greenhouse, especially constructed for citrus-canker investigations, and situated near Washington, D. C. This paper gives findings regarding the invasion of the fruit tissues by the canker organism (*Pseudomonas citri* Hassé), the rate of multiplication of the bacteria in these tissues, and the resultant growth reactions of the fruit. In interpreting results and drawing deductions therefrom one must bear in mind that the environmental conditions in the greenhouse differ in many respects from those in commercial citrus plantings.

METHOD OF INOCULATION

Most of the data from this investigation are of a quantitative nature, based on the number of visible lesions developing under a given set of conditions when a certain number of puncture wounds are inoculated. In routine puncturing of citrus leaves for such quantitative infection tests it has been found convenient to use a simple punch consisting of 10 or 20 pins or needles stuck through a cork. This method of quantitative testing with punctured grapefruit leaves was used in preference to poured agar plates because of the elimination of irregularities due to the growth on plates of contaminating organisms that have an inhibiting effect on *Pseudomonas citri*.

In the case of young fruit it was found that the puncturing instrument ruptured some of the oil glands. The exuding oil had a tendency to injure a portion of the adjacent tissue and to interfere with a normal infection reaction. A definite test of the matter was made by making a large number of punctures singly with a needle directly into the oil glands, and by making another group of punctures elsewhere on the same fruit and taking care to avoid the oil glands. Similar inoculum of *Pseudomonas citri* was applied to both groups, and the number of infections was noted after the lapse of sufficient time for their full development. (Fig. 1.) The results of such a test are given in Table 1.

It is evident that from the standpoint of careful experimental technic it is necessary to avoid making punctures into the oil glands of the fruit. Puncturing of oil glands was avoided in the various experiments described in this paper by making punctures very carefully, one by one, under a magnifier.

¹ Received for publication Feb. 26, 1929; issued September, 1929.

TABLE 1.—Percentages of citrus cankers resulting on fruit from the inoculation of punctures into and between oil glands

Fruit and variety	Diameter of fruit	Cankers developed at punctures—	
		Into oil glands	Between oil glands
	Mm.	Per cent	Per cent
Ponderosa lemon.....	35	0	80
Otaheite orange.....	25	6	32
Pineapple orange.....	40	0	8

The inoculum was prepared from fresh, vigorously growing *Pseudomonas citri* cultures usually on potato plugs. These were checked closely to guard against diminution of virulence. Each batch was made up so as to be of approximately the same concentration. The inoculum was regularly applied to the fruit on moist cotton or cloth

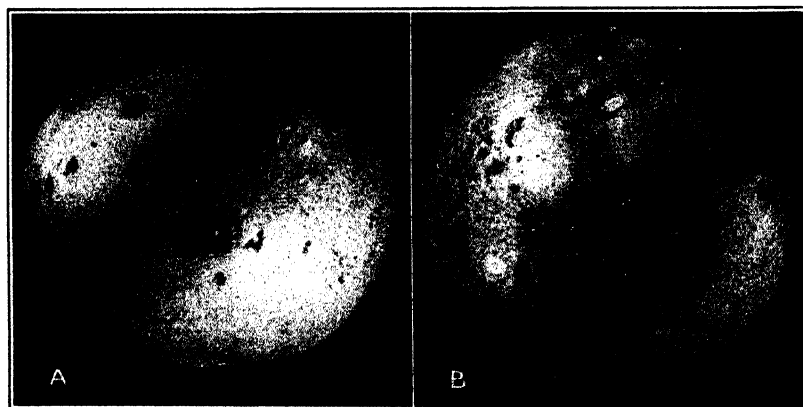


FIGURE 1.—Green grapefruit inoculated with a suspension of *Pseudomonas citri*. On one side (A) a group of punctures was made into the oil glands. On the other side (B) punctures were made between the oil glands. Extensive infection occurred in the latter instance, but none in the former. There are scattered stomatal infections where no punctures were made

swabs. These were left on under wrappings of several folds of paraffin paper, which were removed after a few days.

INFLUENCE OF AGE OF WOUND ON AMOUNT OF INFECTION

To determine what influence the age of the wound at the time of infection has on the percentage of canker lesions that will develop, several groups of 50 punctures between oil glands were made on susceptible young fruits at four different times, over a period of 50 hours. Until the end of the 50-hour period the fruits were left dry and unwrapped. At that time inoculum of the same concentration was applied simultaneously to all groups of punctures on all fruits. The results are given in Table 2.

Evidently wounds as much as 8 hours old when kept dry are not very likely to become infected. Only 3 out of 13 fruits developed any infection through wounds of this age, and such infection was only

one-tenth to one-fifth as great as developed through fresh wounds on the same fruits. These three and one other fruit are the only ones in this test that developed stomatal² infections at apparently uninjured places. The fruits with stomatal infections were the youngest ones in the test, as evidenced by their comparatively small sizes for their respective varieties. All of this might indicate a state of greater tissue susceptibility for these fruits; but other fruits showing equal or higher percentages of infection for freshly made wounds failed to develop any infection in the wounds that were 8 hours old.

TABLE 2.—Percentages of green-fruit punctures which developed cankers after being kept dry for different periods before inoculation

Fruit and variety	Diameter of fruit	Infection percentages, when the wounds dried for—				Number of stomatal infections per fruit
		Less than 1 hour	8 hours	26 hours	50 hours	
	<i>Mm.</i>					
Pineapple orange.....	46	20	0	0	0	0
Do.....	55	4	0	0	0	0
Ruby orange.....	38	8	0	0	0	0
Otaheite orange.....	20	20	2	0	0	3
Do.....	25	80	0	0	0	3
Do.....	36	12	0	0	0	0
Duncan grapefruit.....	42	20	4	0	0	1
Ponderosa lemon.....	21	44	6	0	0	5
Do.....	55	33	0	0	0	0
Key lime.....	30	48	0	0	0	0
Rangpur lime.....	40	50	0	0	0	0
Citron.....	34	2	0	0	0	0
Do.....	39	70	0	0	0	0

In another test the fruit was kept moist from the time of puncturing until inoculated. In this case infection occurred readily in wounds 24 hours old, but the percentages were lower than when the wounds were freshly made.

Perhaps here, as in the case of oil-gland punctures, the injury or killing of one or more layers of cells renders the substratum unfavorable for the development of infection. A fresh wound gives ready access to cells still living, but after the lapse of a few hours the progressive dying of exposed cells may present an obstacle to infection.

Under natural conditions the citrus-canker bacteria are disseminated mainly in water, and some of the greatest outbreaks of infection have occurred after violent storms that injured foliage and fruits. It might be inferred from these experimental tests that injuries such as thorn scratches, limb rubs, sand scurfs, or insect punctures or bites, occurring during dry weather, with the lapse of some hours before precipitation, would lead to comparatively little infection.

INFLUENCE OF STRENGTH OF INOCULUM ON AMOUNT OF INFECTION

Tests were made of the effect of using very strong as compared with weaker grades of inoculum on wounded citrus fruits selected so as to be as nearly as practicable of the same size. The strongest inoculum, indicated as 1/1, was made by using one 3-day-old potato-cylinder

² For convenience the term "stomatal infections" has been used to indicate the infections arising at points not wounded, although it is recognized that some of these may have been in reality at unrecognized wounds.

culture of *Pseudomonas citri* in 200 c. c. of water. Dilutions of 1 in 20 and 1 in 400 were made from this. In Table 3 are given the results of one test carried out in this way. Others gave similar results.

TABLE 3.—Percentages of wound infection of citrus fruits resulting from the use of various dilutions of inoculum

Fruit and variety	Diameter of fruit	1/1 inoculum		1/20 inoculum		1/400 inoculum	
		Fruits	Average infection	Fruits	Average infection	Fruits	Average infection
		Number	Per cent	Number	Per cent	Number	Per cent
Otaheite orange.....	Mm. 21-27	4	38	2	0.5	2	0.5
Parson Brown orange.....	34-37	1	25			1	2
Double-flowered orange.....	43	1	20			1	5
Chinese lemon.....	46	1	10	1	2		

The strongest inoculum produced the highest percentages of infection, and the resulting cankers appeared first and reached full size soonest. The same inoculum applied at the same time to punctured grapefruit leaves gave 100 per cent infection from the 1/20 dilution and 38 per cent from the 1/400 dilution. In other tests punctured fruits of grapefruit have only rarely exceeded 50 per cent infection even with inoculum as strong as the 1/1 used in this test. This indicates that very much stronger inoculum, approaching 400 times as strong, is required to produce a given amount of infection on wounded fruits than on wounded leaves of grapefruit. Similar findings have been made for commercial oranges, lemons, and limes. In natural outbreaks of canker in orchards, leaves seem to be much more readily infected than fruits.

These points are further shown in a test with Otaheite orange leaves and fruits using strong and weak inoculum, prepared as indicated above, of 1/1 and 1/400 strengths. (Table 4.) In recording results four stages of development of a canker spot are recognized: (1) Watery stage, the first distinctly visible water-soaked appearance; (2) pimple stage, small definite spots very slightly, if at all, raised; (3) blister stage, spots distinctly raised and enlarged but not broken open; and (4) erumpent stage, corky development approaching fully formed cankers. Table 4 indicates by stages the rate of development of the canker lesions as well as the percentages of infection on Otaheite oranges. The development throughout was somewhat slow because of rather low night temperatures in the greenhouse in the winter, when this test was made.

With each strength of inoculum the canker development was greater and faster on mature leaves than on either size of fruit, and on the smaller and younger fruits than on the somewhat larger and older ones. The strong inoculum brought about a quicker development of canker than did the weak inoculum on leaves and on both sizes of fruits. Comparing effects of 1/1 inoculum on fruits with those of 1/400 inoculum on the mature leaf, it is seen that the increase of four hundredfold in strength of inoculum was not sufficient to cause infection on these green fruits comparable with that produced on the leaf by the weaker strength of inoculum.

TABLE 4.—Percentages of infection and rate of development of cankers on punctured fruits and leaves of Otaheite orange, following the use of strong and weak inoculum

Days after inoculation	Strong inoculum, I/1					
	Mature leaf		Small fruit (31 mm.)		Large fruit (44 mm.)	
	Per cent infected	Stage	Per cent infected	Stage	Per cent infected	Stage
2.....	0		0		0	
5.....	0		0		0	
7.....	20	Pimple.....	0		0	
9.....	60	Blister.....	0		0	
15.....	(a)		(a)		(a)	
19.....	100	Erumpent.....	(b)	Watery.....	(b)	Watery.....
26.....	100	do.....	10	Erumpent.....	(b)	Do.....
34.....	100	do.....	20	do.....	4	Pimple.....
43.....	100	do.....	20	do.....	(c)	
50.....	100	do.....	40	do.....		

Days after inoculation	Weak inoculum, I/400					
	Mature leaf		Small fruit (34 mm.)		Large fruit (47 mm.)	
	Per cent infected	Stage	Per cent infected	Stage	Per cent infected	Stage
2.....	0		0		0	
5.....	0		0		0	
7.....	0		0		0	
9.....	15	Blister.....	0		0	
15.....	75	do.....	(b)	Watery.....	0	
19.....	80	do.....	(b)	do.....	0	
26.....	85	Erumpent.....	(b)	do.....	0	
34.....	85	do.....	(c)		(c)	
43.....	90	do.....				
50.....	90	do.....				

* No record made.

† Few cankers.

* Fruit dropped.

RATE OF MULTIPLICATION OF PSEUDOMONAS CITRI IN FRUIT LESIONS

The method used for estimating the relative numerical increase of bacteria in inoculated fruit punctures was as follows: Punctures were made in numerous groups of 10 each on the fruit to be tested. Inoculation was made with cotton swabs in the usual way, the inoculum being strong enough to insure infection. Later at various time intervals a given number of the groups of punctures, regularly 6 comprising a total of 60 punctures, were removed with a flamed disk cutter in such way as to include the full thickness of the peel. These 6 uniformly cut disks were thoroughly teased out in 20 c. c. of sterile water. A portion of the resulting bacterial suspension, designated as d/1, was further diluted 1 in 20, and the dilutions were continued in the same ratio as far as might seem requisite. These several dilutions were then used to inoculate freshly made punctures on grapefruit leaves, regularly using 200 punctures on each of five leaves on each of two plants, making 2,000 punctures for a test of each dilution of each sampling. Records were made in due time of the number of cankers developing on the test leaves. The senior writer

has previously estimated³ by this method that 2 to 4 infections developing in 2,000 punctures give evidence of something like 30 viable organisms per cubic centimeter of inoculum. This ratio of approximately 10 organisms per cubic centimeter of inoculum to 1 infection per 2,000 punctures holds very regularly up to about 300 infections per 2,000 punctures. Table 5 gives the results of one such test.

TABLE 5.—Increase of *Pseudomonas citri* in a 34-mm. Otaheite orange fruit, as evidenced by the number of infections per 1,000 punctures developing on grapefruit leaves from inoculum prepared from samples taken at stated times from the orange fruit

Days after inoculation	Infections per 1,000 punctures developing from various dilutions of inoculum from samples from orange fruit				
	d/1	d/20	d/400	d/8,000	d/160,000
0.....	0	0	0		
2.....	80	6	0.5	0	
5.....	160	3	.5	0	0
7.....	1,000	975	55	8	0.5
9.....	1,000	500	14	0.5	0
15.....	1,000	1,000	1,000	100	28
19.....	1,000	1,000	1,000	75	11
26.....	1,000	1,000	1,000	57	8

Evidently there was a very definite and considerable increase during the first 15 days in the number of bacteria in the sampled fruit punctures. From the fifteenth to the twenty-sixth day there was no evidence of continued increase, but there was a suggestion of possible decline in numbers. Tests made on the fifth and ninth days seem to be erratically low. Comparing the showing of the second day with that of the seventh, and the latter with that of the fifteenth, and noting the dilution of the original inoculum required to produce about the same effect on the test leaves, it is seen that there was an increase of perhaps two to four hundredfold of bacteria in the fruit wounds during each of these two periods. This means about eight generations of bacteria, if a regular geometrical rate of increase is maintained, during a 5-day period in the first case and during an 8-day period in the second. With the probability of considerable slowing down or actual reaching of a standstill two or three days before the fifteenth day, one may presume that most or all of the increase for the second period was also during a 5-day period.

The Otaheite orange fruit used in this test was the 34-mm. small fruit receiving the weak 1/400 inoculum as shown in Table 4. The test was run at a somewhat low range of greenhouse temperature during the winter. This fruit did not give external evidence of infection until the fifteenth day, when there was hardly more than an indefinite watery appearance, and it did not develop more decided symptoms before dropping after the twenty-sixth day.

The sampling taken on the fifteenth day in the d/160,000 dilution gave infection of 28 out of 1,000 or 56 out of 2,000 wounds on grapefruit leaves. This would indicate about ten times 56 or 560 organisms

³ FULTON, H. R. DECLINE OF *PSEUDOMONAS CITRI* IN THE SOIL. Jour. Agr. Research 19: 207-223. 1920.

per cubic centimeter for the inoculum used. The estimate for the d/1 inoculum would, therefore, be 160,000 times 560 or 89,600,000 organisms per cubic centimeter. If this is regarded as an even 90,000,000, the 20 cubic centimeters of this inoculum would have an estimated bacterial content of 1,800,000,000. These were from 60 inoculated fruit punctures, only a few of which showed the first suggestion of canker development. This would indicate an average of about 30,000,000 organisms in each incipiently infected wound at the end of the 15-day period. Tests with samples from similar spots made immediately after inoculation gave negative results even in the d/1 dilution by amethod that should have shown the presence of as many as 30 organisms per cubic centimeter of inoculum, corresponding to 10 organisms from each sampled puncture. This indicates an increase ratio of something like 1 to 3,000,000.

Tests parallel to those shown in Table 5 for the small Otaheite orange fruit were made for the other five inoculated fruits and leaves shown in Table 4, except that samplings were not begun until the ninth day for the strong-inoculum series, and additional samplings were made on the thirty-fourth and fiftieth days for parts that had not dropped previously.

In an earlier test made in September similar strong inoculum was used on a 34-mm. Otaheite orange fruit and on a mature leaf of the same plant, and three tests for bacterial increase were made on these at intervals during the first eight days. The data secured from this experiment seem to be concordant with the later results and are included in Figure 2.

The curves of Figure 2 were plotted to represent relative rates of increase of the bacteria in these six inoculated parts. The points on these curves were determined as follows: Relative estimates of the number of bacteria per infected puncture were made for each sampling by multiplying the dilution denominator by the number of infections produced per 2,000 leaf wounds; by multiplying this product by the factor 10 (average number of organisms per cubic centimeter of

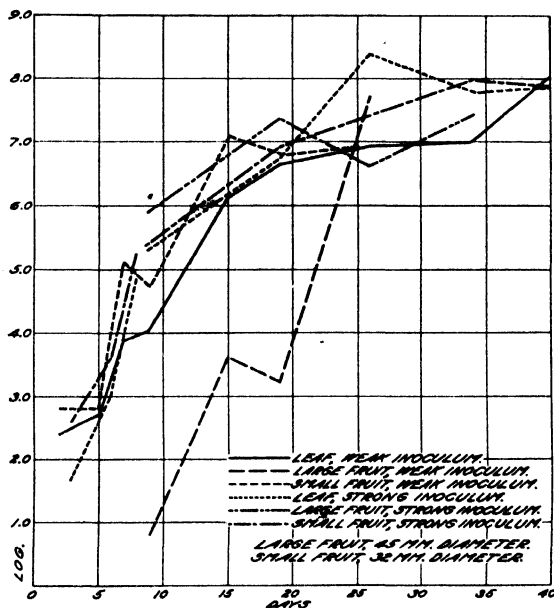


FIGURE 2.—Comparative rate of increase on a logarithmic basis of *Pseudomonas citri* in the tissue of large fruit, small fruit, and leaf of Otaheite orange following inoculation with strong (1/1) and weak (1/400) inocula. The breaks in the curves for the small fruit (strong inoculum) and the leaf (strong inoculum) indicate that the data were taken from two separate tests

inoculum to produce 1 infection per 2,000 leaf wounds), and by 20 (the number of cubic centimeters of inoculum); and by dividing the final product by 60 (the number of fruit punctures in the sample). An example of such a calculation is given on page 407.

To secure the greatest possible reliability for the estimates, two calculations were made for each sampling based on the results of two dilutions of inoculum selected as giving readings as nearly as possible within a range of 20 to 300 infection counts per 2,000 leaf punctures. The two estimates so obtained were averaged for the final estimate. The resulting number range from small to very large. To keep the graph within convenient size, the logarithms of the numbers were plotted rather than the numbers themselves. Since the multiplication of bacteria tends to be in geometrical progression, the plotting of logarithms gives a proper representation of such increase.

The indication from the increase curves of Figure 2 is that the bacteria multiplied with fairly equal rapidity in five of the six tests despite differences in host tissue and in strength of inoculum. The exception is the test of the larger fruit with the weak inoculum. Here there apparently was a slow start, followed by a very rapid increase to a maximum somewhat above that reached by four of the other five tests in the same 26-day period. The significant thing is that bacterial increase within the tissue should have been at so nearly the same rate while the development of visible lesions in the same material was so markedly different in both rate and extent of development, as has been shown in Table 4. For instance, on the ninth day for the strong inoculum neither of the fruits gave visible evidence of infection, while the leaf showed 60 per cent of the wounds at the blister stage. Inoculum prepared from samples of all three parts was of about equal potency on wounded grapefruit leaves. In the case of the three weak-inoculum tests there was an initial lag, which was overcome by the fifteenth day in two cases and by the twenty-sixth day in the third case. Generally speaking, approximately maximum development of bacteria was reached in about 15 days.

The test was repeated, using Otaheite orange of three sizes (40, 31, and 22 mm. in diameter) in comparison with mature leaves, and the same general results were obtained.

These experimental results raise a question as to what constitutes or measures susceptibility or immunity to citrus canker. Judging by external appearances as recorded in Table 4, one would say that the mature leaves of Otaheite orange are much more susceptible to wound infection than a 31-mm. fruit. But five samplings from the ninth to the fifty-sixth day gave indications of somewhat greater numbers of bacteria in the fruit than in the leaf for four of the five samplings. Even more striking is the comparison between the mature leaf and the small fruit receiving the weak inoculum. The development of an externally apparent canker is really a growth reaction of the host tissues. The presence of the causal organisms in sufficient numbers is a requisite for such development; but apparently under certain conditions the bacteria may be present in equal maximum number after having been equally active in multiplying within the tissue, and yet produce little or no canker reaction. In such cases the absence or weakness of canker development, usually taken to indicate resistance to the disease, does not seem to be due to any restraining

of bacterial activity, at least so far as their ability to grow and reproduce is concerned. This phenomenon constitutes what may be termed "quasi resistance" or "quasi immunity." This places emphasis on the importance of the reaction capacity of the host tissue rather than on the ability of the bacteria to flourish within the tissue as a determining factor in the production of canker lesions. The weak inoculum produced less visible effect than that four hundred times as strong (Table 4), but the results plotted in Figure 2 show that by the fifteenth day in two cases out of three the weak inoculations had reached approximately the same numerical level for bacterial invasion as the strong. While it might be assumed that a certain threshold number of bacteria must be present, or a certain quantity of bacterial metabolic activity must go on, in order to provide the proper stimulus for canker reaction in a given host-plant tissue, the present data indicate that the citrus tissue may be in a relatively nonresponsive condition without apparently offering any hindrance to *Pseudomonas citri* development, but in which the visible canker reaction is considerably limited or does not take place. Host tissues in different conditions of responsiveness may require different amounts of bacterial stimulus for a given effect, in which case the same amount of bacterial stimulus would be expected to produce different effects.

RATE OF MULTIPLICATION OF *PSEUDOMONAS CITRI* IN GREEN FRUITS OF VARIOUS CITRUS VARIETIES

Using the method already described, tests were made on green citrus fruits of various varieties and various sizes. These tests were made during the summer months, and the higher range of temperature gave a more rapid rate of multiplication of canker bacteria than that shown in Figure 2. The samplings were made at more frequent intervals. Conditions were favorable for blue-mold rot, and the fruits often became infected through the sampling wounds in spite of all precautions. Whenever blue-mold rot set in, tests for viable canker bacteria gave negative results. Even before the sampled portion actually rotted there was a considerable reduction in the number of bacteria. The effect of blue-mold rot on the persistence of the canker organism is considered further in another section of the paper.

Logarithms to the first decimal place have been calculated to represent the average rate of increase of bacteria per inoculated puncture, and the results are given in tabular form in Table 6 for three separate experiments.

The first samplings made after inoculation gave somewhat irregular results. If such sampling is made immediately after the inoculum is applied, the showing for viable bacteria is abnormally high because of inclusion of the residue of inoculum. If made after about an hour, when the fruit has become surface dry, the showing is many times lower or even negative. Since the rate of drying and consequent dying of superfluous bacteria is very variable, the results obtained for starting figures are irregular and sometimes abnormally high. Usually only one fruit of a series was sampled at the time of inoculation, and the estimate obtained was assumed to hold for all in the series.

TABLE 6.—*Relative logarithmic numbers of citrus-canker organisms estimated for infected punctures of green citrus fruits of various diameters and of leaves sampled at different times after inoculation, indicating the progressive numerical increase on a geometrical basis*

Experiment A				Experiment B				Experiment C			
Days after inoculation				Days after inoculation				Days after inoculation			
Ponderosa lemon				Ponderosa orange				Ponderosa orange			

For the three Ponderosa lemons the rate of increase was much the same up to the fifth day, when the smallest one began to show a decline, the fruit being invaded by *Penicillium*. The 87-mm. fruit was similarly affected after the fourteenth day. This largest fruit did not develop any external evidence of canker reaction during the 24 days. The 62-mm. lemon developed 50 per cent of fully erumpent cankers during the test. The smallest (37 mm.) fruit showed 10 per cent of erumpent spots before its premature loss, and undoubtedly would have shown the greatest amount of canker reaction if it had persisted.

The two sizes of Parson Brown oranges and the two of Pineapple oranges showed definite increases to about the same general level. Unfortunately, three of these four fruits succumbed to blue-mold rot after the fourth day and before visible cankers could be expected. The remaining 37-mm. Pineapple orange developed 5 per cent erumpent cankers during the first 10 days. The 37-mm. Satsuma orange did not develop any external evidence of infection during the 19-day period. Field observations in canker-infested territory have shown the very high resistance amounting almost to immunity of Satsuma fruits to natural infection. In the present experiment the rate of increase in Satsuma fruit was fully as rapid as in the Pineapple orange of the same size, the latter developing normal symptoms of canker and the former none. The invasion through wounds and the subsequent multiplication of canker bacteria seem to have been closely parallel. One variety reacted in such a way as to form cankers, but the other variety did not visibly react. The grapefruit leaf in the same experiment gave lower tests in early stages than the orange fruits, but the maximum finally reached was approximately the same. The leaf by the thirteenth day had developed cankers of the blister stage at 100 per cent of the wounds.

The last section of Table 6 (experiment C) shows very rapid increase during two days in wounded fruits of key lime and Walters grapefruit, and the increase in wounded grapefruit leaf is given for comparison. Unfortunately, the two fruits were soon lost from blue-mold rot. The grapefruit leaves showed 90 per cent of visible canker in the blister stage on the tenth day. The possibility of wound infection without external reaction is thus demonstrated for a representative series of green citrus fruits. With increase in size of fruit beyond a certain point the development of external visible lesions is hindered, but the bacteria multiply practically as rapidly as when typical cankers are formed.

RATE OF MULTIPLICATION OF *PSEUDOMONAS CITRI* IN MATURE FRUITS OF
VARIOUS CITRUS VARIETIES

Some of the inoculated fruits used in previous tests were approaching full size, but the peel was still green. It seemed desirable to test fruit in a more matured condition. Tests were made on fully colored fruit still attached to the trees in the greenhouse. The results are given in Table 7. A comparison with Table 6 shows very little difference in rate of increase between green and mature fruit. As mature fruit soon became infected with blue-mold rot, the tests ended prematurely.

TABLE 7.—Relative logarithmic numbers of citrus-canker organisms estimated for each infected puncture of ripe citrus fruits sampled at different times after inoculation, indicating the progressive numerical increase on a geometrical basis

Experiment A				Experiment B		
Days after inoculation	Pineapple orange	Duncan grapefruit	Ponderosa lemon	Days after inoculation	Pineapple orange	Ponderosa lemon
0.....	2.8	2.8	2.8	0.....	1.3	0.9
1.....	7.9	6.0	7.7	1.....	4.2	3.9
2.....	6.8	7.4	6.6	2.....	5.2	6.2
3.....				3.....	5.0	7.3
5.....	5.3	2.5	5.4	6.....	2.3	4.0

Mature fruits of orange, grapefruit, and lemon from the market were tested at two different times. In one instance the fruit was lost from decay after the fifth day; in the other it was held over a 28-day period and sampled every five days. In both tests the orange and the lemon gave negative results at every sampling after the first, which was made just after inoculation. The grapefruit in each case gave low estimates on the fifth day, indicating persistence with little if any multiplication. In the longer test the same condition was shown by the grapefruit on the tenth, fifteenth and twenty-first days, with negative results on the twenty-eighth day, the last testing. In still another experiment a mature grapefruit from the market was inoculated through wounds in the usual way, and 74 days later a test indicated something like 32,000 bacteria per puncture. This fruit had been held without intervening sampling and had not developed external lesions.

There is apparently a very marked difference in the behavior of the canker organism following inoculations in the peel of mature fruit after removal from the tree as compared with its behavior in the peel of mature fruit still on the tree. Possibly changes in the physiological condition of the fruit resulting from its removal from the tree are responsible for this difference. It is to be noted that in the cases of *Diplodia* and *Phomopsis* stem-end rots the advance of rot into the peel normally does not occur until some days or weeks after the fruit is removed from the trees, although there is abundant evidence that incipient infection already exists. Here the senescent changes in the peel favor the development of fungi having saprophytic tendencies; it is not inconsistent to presume that these changes would in equal degree hinder the development of an organism having definitely parasitic habits like *Pseudomonas citri*.

PERSISTENCE OF BACTERIA IN OLD CANKERS ON MATURE FRUITS

The question arises about the viability of *Pseudomonas citri* in typical canker lesions of mature fruits that originated while the fruit was young and green. In the greenhouse experiments certain fruits were inoculated while young and developed typical canker lesions. Six to seven months later, when these fruits were fully mature, tests were made on punctured grapefruit leaves in the usual way for evidence of viable canker bacteria. The test included two fruits of Pineapple orange, three fruits of Otaheite orange, two fruits of Pon-

derosa lemon, two fruits of Rangpur lime, one fruit of Temple orange, and one fruit of Duncan grapefruit. In every case the results were negative. (Fig. 3.)

From time to time fruits with well-developed cankers have been intercepted by quarantine inspectors and submitted for diagnosis. The obtaining of viable *Pseudomonas citri* from such specimens has been uncertain, although in a few instances successful cultures of the canker organism have been made from such material. (Fig. 4.) Infected fruits were too limited in number for a definite determination of the time limits of viability of the organisms in the lesions. This doubtless varies greatly with conditions.

EFFECT OF PENICILLIUM ROT ON VIABILITY OF PSEUDOMONAS CITRI

It was noted earlier in this paper that development of *Penicillium* rot in a fruit under test is regularly followed by a decrease in the number of viable canker bacteria recoverable. To test the matter definitely, mature orange fruits on the tree were puncture-inoculated at several locations with the canker organism. One day later *Penicillium digitatum* was introduced at one place on the fruit. Two days after this, when the softening from the blue-mold rot was apparent, tests were made from groups of punctures very near the advancing edge of the rot, but where the tissue had not yet softened, and also from punctured areas on the opposite side of the fruit. In one experiment the average of five tests from near the edge of the rotted area gave 5 infections per 1,000 grapefruit-leaf punctures for the d/1 inoculum, and a similar average for material from the opposite side of the fruit was 975 infections per 1,000 test punctures for a similar dilution of inoculum. In a second experiment samples taken from areas recently invaded by *Penicillium* gave negative results, while material taken at a distance gave an average of 403 infections per 1,000 test punctures.

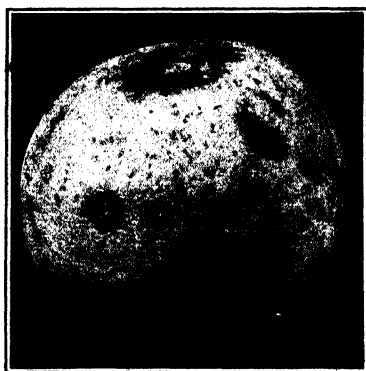


FIGURE 3.—Canker lesions on a mature Pineapple orange, the result of inoculation of the young fruit six months previously. These old cankers did not give evidence of containing viable *Pseudomonas citri* when culture and inoculation tests were made

INFLUENCE OF SIZE OF FRUIT ON DEVELOPMENT OF CANKER

In previous tests estimates were made quantitatively for the presence of the canker bacteria in the host tissues, and estimates of their numbers were made regardless of whether there were externally apparent lesions. Such testing requires much time and material. In ordinary practice reliance is put on the development of externally visible lesions to determine the fact and extent of infection. In the experiments that follow, this observational method was used to determine the range of fruit size or age in which infection is possible, either through wounds or without wounding, and the size of fruits giving the best development of lesions.

The method used may be illustrated by the following experiment: Four Homosassa oranges of approximately the same size, averaging 30 mm. in diameter, were punctured carefully between the oil glands, 50 punctures in each group, inoculated with standard inoculum on a swab, and wrapped. About 7 to 10 days later a new group of punctures was made in a different location on the same fruits, inoculum was applied, and the fruits were remeasured. The same procedure was followed until five successive tests had been made on these fruits, the last being when their average size was 50 mm. Close records were kept of the starting of visible infection at any group of wounds, or on the adjacent unwounded but inoculated surface.

In this test the average infection at the outset, at 30 mm. average size, was 47 per cent of erumpent lesions. The greatest average percentage of infections for any inoculation was 82 per cent at the

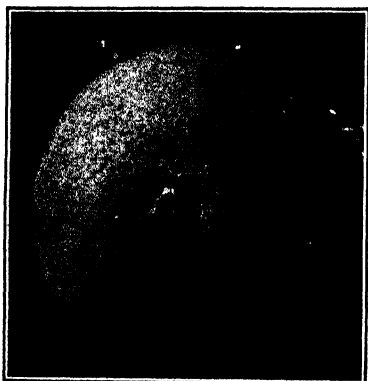


FIGURE 4.—Orange infected with canker, intercepted at San Francisco in the baggage of a passenger from Japan. Cultures or inoculation material from old lesions on such mature fruits may or may not give evidence of viability of *Pseudomonas citri*

36 mm. average size, and these lesions reached a greater size than the others. At an average size of 48 mm. the lesions were still fairly numerous, averaging 41 per cent, but they were quite small, and none became erumpent. At 50 mm. average size there was only 9.5 per cent of infection, and the lesions were barely discernible as such. It thus appears, as judged by ordinary standards, that Homosassa oranges, under the conditions of this test, manifested greater susceptibility to canker infection through wounds at a size of about 36 mm. (nearly $1\frac{1}{2}$ inches) in diameter than at smaller or larger sizes. The upper size limits of susceptibility to wound infection seemingly had been almost reached at the end of the test at an average size of 50 mm. (2 inches).

In this test, fruits developing stomatal infections were more closely limited in size range, the smallest fruits showing any infection being the 29 mm. size and the largest 35 mm., with an average size of 32 mm. and an average of eight infections per fruit, all well developed. The beginning size for stomatal infections on this variety is not indicated by this test, and the optimum size is very likely smaller than the initial sizes used. Since inoculations were repeated three or four times after the fruits had passed the 35 mm. size, it may be safely concluded that this is about the upper limit of susceptibility for stomatal infection of this variety under the conditions of this experiment.

In the same general way tests were made on a considerable number of varieties of green citrus fruits in the greenhouse. (Fig. 5.) Since the supply of such fruits was limited, it was not usually possible to select a group of the same size for inoculation at the same time. Inoculations were therefore made on fruits of any suitable size, proper measurements and records being made. It is recognized that size is by no means an exact indication of age, since individual fruits

may grow at different rates even when of the same variety, and the different varieties and classes of citrus fruits vary much in rate of growth and maximum size attained. This testing of varieties extended over three seasons, and naturally the various fruits used were subjected to a variety of growth conditions. An attempt was made to make inoculations as uniformly as possible, and with inoculum strong enough to produce a maximum of infection. Parallel inoculations were always made on punctured grapefruit leaves and always yielded practically 100 per cent of infection. Difficulty was experienced in getting records on fruits smaller than about 18 mm., because the injury caused by the puncturing and the covering of moist wrappings seemed to induce dropping. Records of infection were made after sufficient time had elapsed for infection to reach a

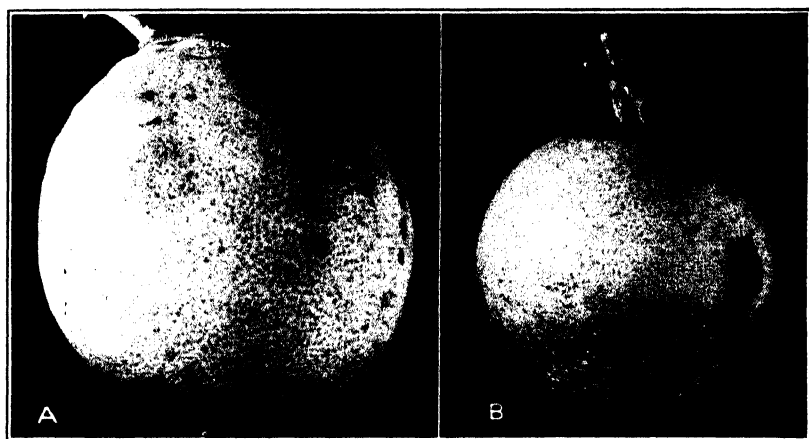


FIGURE 5.—A.—Ponderosa lemon. Five rows of 10 punctures each (partly showing) were made and inoculated when the fruit was 50 mm. in diameter. These developed 82 per cent infection. The circular group of 50 punctures at the left was made and inoculated when the fruit had reached a diameter of 65 mm., but no infection resulted. B.—Navelencia orange. The circular group of 50 punctures at the right side of the fruit was made and inoculated when the fruit was 28 mm. in diameter, and developed 30 per cent infection at wounds. There were also a few stomatal lesions. Another group of 50 punctures (near the lower side of the fruit) was made and inoculated when the fruit had reached a diameter of 43 mm. These did not become infected.

maximum, and distinction was made between wound and stomatal infections.

Table 8 gives the summarized results of these tests with particular reference to the relationship of fruit size to development of infection. In interpreting these results it must be borne in mind that the lower limits of infectable range could not be definitely determined because of the difficulty in getting very small sizes to persist after being inoculated. The poor condition of some that remained may have interfered with normal development of infection. If the fruits were not punctured, more of them remained attached, and so the test range could be carried lower for stomatal than for wound infections. It is noteworthy that the sizes that gave the greatest amounts of visible infection were usually at some distance from either the upper or the lower limits of the range tested, when any considerable number of tests were made for any variety. Toward the upper limits of infection the reaction lessened progressively from visible excres-

cences to mere external yellowing and finally to slight internal discoloration. It must be emphasized that the results in Tables 8 and 9 are not finally conclusive, and caution must be exercised in making any general deductions from them. In some instances the numbers of fruits tested were too low for reliable results. Numerical estimates based on the inoculated leaf puncture method of testing have shown the active invasion of fruits of Valencia orange to be about equal to Pineapple orange in one specific experiment, and that of Washington Navel to be distinctly less. In the same way invasion of fruit of Royal grapefruit was proved to take place, but in a less degree than in Marsh grapefruit. A single test to determine invasion of fruit of Nagami (oblong) kumquat gave negative results under rather unfavorable conditions for the test, due to the usually quick rotting of wounded kumquats.

TABLE 8.—Results of inoculating fruits of various citrus varieties with *Pseudomonas citri*, showing the sizes most readily infected

Fruit and variety	Data concerning wound infections					Data concerning stomatal infections				
	Tests	Size range tested	In-fected fruits	Size range in-fected	Optimum size for infection	Tests	Size range tested	In-fected fruits	Size range in-fected	Optimum size for infection
	Number	Mm.	Number	Mm.	Mm.	Number	Mm.	Number	Mm.	Mm.
Pineapple orange	54	15-58	36	19-58	40	60	8-58	10	22-43	31
Homocassa orange	30	17-54	23	17-54	36	36	17-54	3	29-35	30
Parson Brown orange	34	15-61	20	21-57	33	34	15-61	3	22-26	22
Mediterranean Sweet orange	11	21-49	4	21-48	25	15	16-49	1	21	21
Valencia (Lue) orange	9	25-49	0			9	25-49	0		
Navelencia orange	8	25-54	3	25-32	27	8	25-54	2	28-32	28
Ruby orange	4	38-58	1	38	38	4	38-58	0		
Washington Navel orange	3	18-49	0			3	18-49	0		
Satsuma orange	37	18-51	12	18-40	28	50	11-51	3	28-35	33
King orange	19	20-38	6	22-38	30	20	20-38	0		
Tangerine orange	17	16-42	2	23-28	28	22	11-42	1	24	24
Temple orange	4	38-60	0					0		
Otaheite orange	39	9-70	22	14-47	24	46	11-70	3	20-25	25
Double-flowered orange	14	25-48	6	33-46	42	17	19-48	0		
Myrtleleaf orange	13	16-45	3	25-36	30	13	16-45	0		
Willowleaf orange	3	30-40	1	30	30	3	30-40	0		
Duncan grapefruit	28	25-66	11	25-53	40	33	8-66	8	25-42	38
Walters grapefruit	11	28-65	3	28-48	48	13	15-65	2	40-48	40
Royal grapefruit	3	28-46	0			3	28-46	0		
Eureka lemon	9	30-45	3	33-36	33	11	21-45	0		
Lisbon lemon	10	21-40	1	38	38	9	21-40	0		
Kenedy lemon	10	23-38	2	27-35	31	11	17-38	0		
Villafraanca lemon	6	27-45	1	27	27	7	27-45	0		
Lamb lemon	5	28-48	0			3	18-49	0		
Chinese lemon	19	30-53	8	30-46	36	19	38-53	1	35	35
Rough lemon	8	21-33	0			14	18-33	0		
Ponderosa lemon	45	17-92	25	21-88	35-75	54	11-92	9	21-70	56
Key lime	20	14-31	13	17-30	26	32	9-31	3	20-28	24
Rangpur lime	36	18-55	22	18-48	27	51	8-55	3	22-24	23
Sylhet lime	16	15-35	6	19-33	22	16	15-35	1	19	19
Suntara lime	8	18-32	6	18-29	23	8	18-32	1	21	21
Bearss lime	11	17-37	7	17-36	20	11	17-37	0		
Kusale lime	18	11-32	3	18-32	20	22	7-32	0		
Dominican lime	18	14-36	3	16-26	21	21	9-36	0		
Wogium lime	2	34-36	0			2	34-36	0		
Tahiti lime	7	15-35	0			10	12-35	0		
Nagami kumquat	17	12-23	0			17	12-23	0		
Citron	9	34-44	3	34-39	39	9	34-44	0		
Citrus excelsa	5	12-28	1	18	18	6	12-28	0		

In Table 9 data from the same tests are used as a basis for quantitative expressions of infection for the various varieties. In the tabulation are included only those fruits that fall within the known infectible range of size for the particular variety. Not all fruits within such range actually developed infection in the tests. Several varieties in which neither wound nor stomatal infection was secured are omitted from this table. However, it is to be supposed that some of these might react under more suitable conditions or with more extended testing. Since the bases on which percentages of infection are calculated are reduced by this attempted elimination of sizes that may be immune, the resulting showing of infection is higher and supposedly a more correct index to the relative susceptibility of the various species. But caution must be exercised in drawing general conclusions from data having the unavoidable limitations of these. It must be recalled that what is here termed "susceptibility" is evidenced and measured by the outwardly visible reaction of the host tissues to the bacterial invasion. It has been shown in other cases that the bacterial invasion takes place just as truly, but the host tissues do not respond sufficiently to show any external effect. The present data do not take into account such invisible invasion.

TABLE 9.—Results of inoculating infectible fruits of various citrus varieties with *Pseudomonas citri*

Fruit and variety	Data concerning wound infections						Data concerning stomatal infections					
	Fruits of susceptible sizes			Infected fruits			Fruits of susceptible sizes			Infected fruits		
	Fruits	Infected		Punctures	Lesions	Punctures with lesions	Fruits	Infected		Lesions	Average lesions per fruit	
		Number	Percent					Number	Percent		Number	Number
Pineapple orange	52	36	69	1,800	614	34	43	10	23	85	9	9
Homossassa orange	30	23	77	1,150	415	36	11	3	27	30	10	10
Parson Brown orange	34	20	59	1,000	176	18	12	3	25	31	10	10
Mediterranean Sweet orange	11	4	36	200	32	16	5	1	20	1	1	1
Navelencia orange	3	3	100	150	110	73	3	2	67	30	15	15
Ruby orange	1	1	100	50	4	8	0	0	0	0	0	0
Satsuma orange	35	12	34	600	99	17	49	3	6	27	9	9
King orange	10	2	20	100	6	6	6	1	17	10	10	10
Tangerine orange	10	2	20	100	6	6	6	1	17	10	10	10
Orangette orange	29	22	76	1,100	221	20	12	3	25	10	3	3
Double-flowered orange	12	6	50	300	56	19	0	0	0	0	0	0
Myrtleleaf orange	10	3	30	150	9	6	0	0	0	0	0	0
Willowleaf orange	1	1	100	50	10	20	0	0	0	0	0	0
Duncan grapefruit	23	11	48	550	157	29	13	8	62	53	7	7
Walters grapefruit	7	3	43	150	45	30	6	2	33	25	13	13
Lisbon lemon	4	1	25	50	26	62	0	0	0	0	0	0
Eureka lemon	5	3	60	150	26	17	0	0	0	0	0	0
Kenedy lemon	8	2	25	100	64	64	0	0	0	0	0	0
Villafraanca lemon	2	1	50	50	13	26	0	0	0	0	0	0
Chinese lemon	18	8	44	400	56	14	9	1	11	2	2	2
Ponderosa lemon	43	25	58	1,250	541	43	39	9	23	71	8	8
Key lime	20	13	65	650	116	18	20	3	15	13	4	4
Rangpur lime	32	22	69	1,100	425	39	11	3	27	9	3	3
Sylhet lime	16	6	38	300	81	27	3	1	33	5	5	5
Suntara lime	7	6	86	300	66	22	4	1	25	1	1	1
Bearss lime	11	7	64	350	52	15	0	0	0	0	0	0
Kusale lime	17	3	18	150	105	70	0	0	0	0	0	0
Dominican lime	17	3	18	150	14	9	0	0	0	0	0	0
Citron	9	3	33	150	43	29	0	0	0	0	0	0
Citrus excelsa	1	1	100	50	3	6	0	0	0	0	0	0

INOCULATION OF FRUIT ON ORCHARD TREES

On account of the vigorous eradication campaign against citrus canker, it has not been possible for the writers to make test inoculations on orchard trees except in one instance. These few trees were outside of a commercial citrus region, in territory in which the eradication work was at the time suspended. The orange trees were seedlings, about 12 years old when the inoculations were made in May. There was already considerable old and fresh canker infection on the leaves of the lower limbs, but none was observed on the fruits. Fruits for inoculation were selected on the topmost branches above the drip from infected leaves. The fruits were punctured between the oil glands, 100 punctures each. Forty fruits were used, ranging in size from 18 to 38 mm., all but four being 25 mm. or larger. Strong inoculum was prepared by teasing well-developed leaf lesions in water until it looked turbid. The inoculum was applied on cotton swabs, and the fruit was wrapped with waxed paper. Punctured leaves were also inoculated on the same tree.

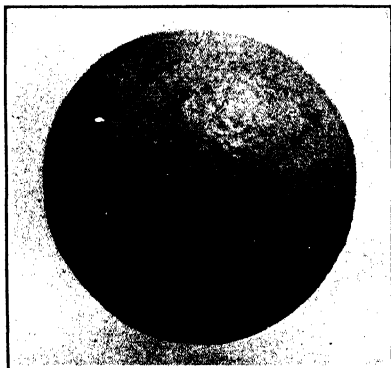


FIGURE 6.—Moist-chamber inoculation on detached green orange, 45 mm. in diameter

After one month, 16 fruits showed infection, all at wounds; 4 of them were in the 25-mm. class and averaged 4 infections per fruit, and 5 were in the 32-mm. class and averaged 3 infections per fruit. The greatest number of infections per fruit was 14 on a fruit of the 32-mm. class; the next was 12 on a fruit of the 38-mm. class; and the next was 6 on a fruit of the 25-mm. class. Of the 16 infected fruits, 7 had 1 infection per fruit. There was no increase in infection during the following three months. No stomatal infection developed on the inoculated fruits or on other fruits on the tree that had a good chance to receive washings from infected leaves. The artificially inoculated leaves developed 100 per cent infection.

While this test was too small in scope for any definite conclusions to be drawn, the behavior of the fruits growing naturally on large trees in this one instance was in general agreement with the experiments in the greenhouse in which green fruits on the trees were inoculated in a similar way.

INOCULATION OF DETACHED GREEN FRUITS IN MOIST CHAMBERS

Since the numbers and varieties of green citrus fruits available for inoculation in the greenhouse were quite limited, attempts were made to broaden the range by using young fruits that had been removed from the trees in Florida and sent to Washington. (Fig. 6.) These were punctured in the usual way, inoculated by dipping in a suspension of *Pseudomonas citri*, and placed in covered dishes lined with moist filter paper, care being taken to avoid too much

moisture. Small fruits, in general less than 20 mm. in diameter, were apt to decay quickly. The larger ones developed canker lesions rather more readily than did those on trees in the greenhouse, but the infection was much less abundant than was induced on leaves of the same variety with the same inoculum. Infection was secured on larger sizes of fruit in the moist chambers than on the trees. There was a tendency for a small proportion of uninoculated fruits, especially very small ones, to form spongy intumescences at the wounds that resembled early stages of canker, but did not develop into typical cankers. Microscopic examination of these showed the absence of bacterial ooze, and platings from them gave negative results for the presence of the canker organism. The inoculated fruits developed similar intumescences in greater numbers, and these always gave abundant evidence of bacterial invasion, both by microscopic examination and by plating, and the cankers in time developed to typical stages.

In the moist chambers there was the same tendency for the larger fruits to develop very slight external cankers, even though the bacteria had multiplied considerably in the tissues. This was determined by microscopic examination, by plating, and by inoculation of punctured grapefruit leaves with dilutions from the suspected lesions.

In one experiment fruits of several varieties and of uniform size for each variety were used in the moist chambers with three grades of inoculum indicated as I/1, I/20, and I/400. Some of the fruits receiving the I/1 inoculum were held in a refrigerator at about 45° F. The results are shown in Table 10.

Where strong inoculum was used the infection was evident sooner and developed in a larger percentage of wounds than where weaker inoculum was used. Marsh grapefruit developed lesions more quickly and extensively for each grade of inoculum than did Royal grapefruit of approximately the same size; and Pineapple orange gave similar evidence of having greater susceptibility than Washington Navel orange. Marsh grapefruit was first in apparent susceptibility, and Rangpur lime was second at both room temperature and in the refrigerator. The final reaction developed with I/1 at low temperature was about equal to that developed with I/20 inoculum on the same kind of fruit at room temperature of about 70° to 75° F., but the time required to develop such reaction was much longer at the lower temperature. In other words, at the lower temperature a much stronger inoculum as well as a much longer time was required to produce a given result.

The relative development of canker lesions on wounded fruits of grapefruit and orange is shown in Table 11. This test was made in moist chambers at room temperature, and three dilutions of inoculum were used. Observations were made over a 51-day period. The averages given are for five fruits in each treated lot. In Table 11 the term "blister" is used for any definite infection that had not become crum-pent, and so it may have included the pimple or even the watery stage.

TABLE 11.—Percentages of canker lesions developing on wounded green grapefruits and oranges held in moist chambers after being inoculated with three dilutions of inoculum

Days after inoculation	Lesions produced on grapefruits (80 mm. average diameter) with dilution of inoculum indicated						Lesions produced on oranges (55 mm. average diameter) with dilution of inoculum indicated					
	I/1		I/20		I/400		I/1		I/20		I/400	
	Crumpent	Blister	Crumpent	Blister	Crumpent	Blister	Crumpent	Blister	Crumpent	Blister	Crumpent	Blister
	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>
14.....	14	26	1	14	0	0	1	1	0	0	0	0
20.....	32	19	4	15	0	2	3	7	0	0.6	0	0.4
28.....	46	19	18	27	6	8	6	21	0	6	0	1
36.....	47	25	28	30	8	14	12	20	1	11	0	3
51.....	47	27	28	30	9	15	12	26	1	18	0	3

The fruits were rather large, having been removed from the trees in August. The total amount of reaction in this moist-chamber test is much greater than developed in greenhouse tests of fruits of comparable size. The usual decrease in reaction with dilution of inoculum is noted. Oranges reacted much less in both amount and degree than did grapefruits. To produce a given effect on these oranges required an inoculum more than twenty times as strong (perhaps fifty to one hundred times as strong) as was required for the grapefruits. This is in keeping with the general results shown in Table 10 for smaller fruits.

In other moist-chamber tests Royal grapefruit developed canker less readily than Marsh, Duncan, and seedling grapefruit or shaddock, and Washington Navel oranges developed canker less readily than

Pineapple, Parson Brown, or Valencia. Fruit of Satsuma orange developed slight infection, even under most severe conditions. The results with King and Mandarin oranges were indefinite because of the early rotting of the fruit. Genoa lemon was on a par with Meyer lemon, and Villafranca and Kenedy reacted even less readily. Key lime fruits developed canker readily. Calamondin and kumquat decayed too quickly to give results.

The moist-chamber tests with wounded fruits were started in April and continued until August, successive lots of fruit being sent two or three times a month. The very smallest fruits used decayed too rapidly for canker to develop. Sizes of 12 to 25 mm. could be held long enough to show at least the beginnings of canker lesions. No infections were noted on fruits of such size range for any of the fruits tested. The smallest sizes to show infection in the various tests were as follows: Seedling grapefruit, 50 mm.; Marsh grapefruit, 54 mm.; Royal grapefruit, 53 mm.; Duncan grapefruit, 50 mm.; shaddock, 80 mm.; Pineapple orange, 40 mm.; Parson Brown orange, 41 mm.; Valencia orange, 38 mm.; seedling orange, 28 mm.; Satsuma orange, 35 mm.; key lime, 30 mm.; Buena Vista lime, 30 mm.; Rangpur lime, 33 mm.; Villafranca lemon, 38 mm.; Genoa lemon, 36 mm.; Kenedy lemon, 38 mm.; Meyer lemon, 38 mm.; Sampson tangelo, 35 mm.; King and Mandarin oranges and calamondin decayed too rapidly for infection to occur.

Not all of the above-named varieties were tested in every series, but those most important commercially were tested certainly once and often twice a month. The inoculum was regularly tested on wounded grapefruit leaves and was always potent enough for 100 per cent infection on these. There was certainly abundant opportunity for smaller sizes to become infected. Stomatal infections were very rare.

Mention has already been made of the practical failure to secure a canker reaction in fully matured fruits of orange, lemon, and grapefruit from the market. The large green fruits of orange and grapefruit in August gave strong reactions in moist chambers. On the whole, reaction was stronger for larger sizes of green fruit in the moist chambers than for similar sizes on the trees, and reaction started at larger sizes in the former case than in the latter. No ready explanation of this shifting suggests itself.

In the moist-chamber tests there was abundant multiplication of bacteria in tissue that did not react visibly, as was shown by using graded inoculum on punctured grapefruit leaves. But extensive tests to determine the rate of such increase and its limits as to size of inoculated fruits were not undertaken in the moist-chamber tests. Often a section cut through the inner peel showed a watery infiltration and a slight change in color, suggesting invasion by the bacteria, which microscopic examination confirmed, even when there was no external reaction.

It has been noted that a larger percentage of infections regularly develop in leaf wounds than in fruit wounds, when the same inoculum is used. It might be supposed that the inoculum penetrates less readily to the interior of the deep fruit punctures than to the open leaf punctures. To test this possibility detached wounded fruits were punctured and immersed in the inoculum under an exhaust so as to

withdraw air and cause thorough penetration. These fruits did not develop in moist chambers more infection than those inoculated in the ordinary way, and in both cases the infection was much less than in leaves inoculated at the same time with the same inoculum.

SUMMARY

A quantitative method is described for estimating from wound inoculations the number of *Pseudomonas citri* present at various stages in the development of canker lesions.

When punctures are made into the oil glands of citrus fruits, infection by *Pseudomonas citri* is seriously hindered. For dependable infection results the oil glands must be avoided in wounding for inoculation tests.

Fruit wounds as much as 8 hours old become infected much less readily than freshly made wounds. If allowed to dry the wounds decrease in infectibility more rapidly than if kept moist.

Weak grades of inoculum produce lower percentages of infection than strong grades, and the resulting cankers begin to show after a longer interval and develop more slowly. With a given strength of inoculum, infection is greater in amount, and in degree on wounded leaves than on wounded fruits of a given citrus species.

The size of the fruit influences the amount and degree of canker development. It is difficult to secure wound infections on very small fruits, partly, perhaps, because of the damaging effect of the wounding and subsequent inoculation treatment. Intermediate sizes, from perhaps 25 to 35 mm. in diameter, show best development of canker lesions. Larger fruits develop lesions less readily. The exact upper and lower size limits of susceptibility vary with conditions and can not be inferred from the data at hand.

The majority of infections occur at visible wounds. The so-called stomatal infections occur most abundantly on somewhat smaller sizes of growing fruits than do wound infections, and stomatal infections fail to develop at an upper size range some 10 mm. less than for wound infections.

Periodical testing of infected wounds indicates that the multiplication of the canker bacteria takes place in about equal degree regardless of the size of fruit up to a stage of full maturity on the trees. Fully ripe fruit inoculated through wounds after removal from the tree did not give evidence of any definite increase of the canker organism. A practically maximum number of bacteria is reached in a few days at ordinary temperatures, after which a rather uniform level is maintained for a considerable time. The multiplication of bacteria in the tissues is independent of the development of external evidences of canker. In many instances where there was no visible symptom of canker the bacteria had multiplied just as freely as where there was normal canker development. Such a condition may be referred to as quasi immunity or quasi resistance.

A maximum development of bacteria in lesions may result from weak as well as from strong inoculum, a slightly longer period being required in the former case.

Development of *Penicillium* rot has a decided inhibiting or killing effect on *Pseudomonas citri* in recently developed lesions in the fruit peel.

Various types and varieties of citrus fruits show differences in the readiness with which either wound or stomatal infections occur. The significance of this in judging relative susceptibility is lessened by the fact that host-tissue reaction may be lacking even when bacterial invasion and development have been at a maximum. The reactive condition of the host tissues is the important factor in canker development.

Tests for persistence of viable canker organisms indicate that they may die out within a period of five or six months, but under certain conditions they may persist for possibly longer periods.

Inoculation tests of limited scope on oranges under orchard conditions indicate general agreement with the findings from experiments in the greenhouse.

Infection tests of green fruits removed from trees and held in moist chambers also confirmed in essentials the findings from the greenhouse experiments. The principal differences were that fruits of the larger sizes developed visible canker lesions rather more readily than did those in the greenhouse tests and that infectibility started at a higher range of size.

At about 45° F. green inoculated fruit in a moist chamber required much stronger inoculum and a much longer time to develop approximately the same visible reaction as similar fruit held at a room temperature of about 70° to 75° F.

THE WOOLLY-KNOT TYPE OF CROWN GALL¹

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INTRODUCTION

This article is a report of experiments which furnish evidence that a bacterial organism, tentatively referred to as the apple strain of the crown-gall organism, is the cause of malformations known as the woolly-knot type of crown gall, on root-grafted apple trees.

REVIEW OF LITERATURE

In 1907 Smith and Townsend (18)³ reported the isolation of an organism from galls on the Paris daisy (*Chrysanthemum frutescens*) which they named *Bacterium tumefaciens*. Later Smith, Brown, and Townsend (17) isolated an organism from malformations on apple roots and referred to it as the apple strain of *Bact. tumefaciens*. Experiments were performed by these workers in an attempt to determine whether this organism was the cause of the malformations on the apple from which it was isolated, but owing to the rather limited extent of these experiments on the apple and to the fact that in many instances the results of the inoculations were negative or the controls became infected, the results were by no means conclusive.

Hedgecock (2) performed numerous experiments dealing mostly with the field control of crown gall and hairy root of the apple tree. Although proof was lacking, he expressed the following opinion:

The period when most apple root grafts become diseased with crown gall is apparently the time when the wounds in the union are being healed by the formation of callus. If the disease could be kept out during this period it is quite probable that little would occur on nursery stock except as it gained entrance later through wounds made in cultivation.

In his recommendations for control he stressed the importance of making "close-fitting root grafts, avoiding blunt ends of the root and scion in the union."

Keitt (3) reported experiments in which grafts were made from carefully disinfected stocks and scions and planted in sterilized soil. He stated that grafts which are not well matched "developed enlargements of the types found in practice." He concluded that the largest overgrowths were produced by misfits in which the lower tips of the scions failed to unite satisfactorily with the stocks, while well-fitted and entirely matched grafts developed little or no excess callus. He noted that the overgrowths occurred where there seemed to be an interference in the stem of the plant.

Melhus (4) stated that "mechanical injury to the root may also lead to the excessive development of fibrous roots from the callus

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² Credit is gratefully extended to R. B. Piper for most of the cultural and inoculation work in these experiments.

³ Reference is made by number (italic) to "Literature cited" p. 449.

formed in the wound. "This condition is not infectious." However, he also reported: "On seedlings * * * most of the so-called hairy root is not infectious, the infectious hairy roots arising from crown galls," and "so-called hairy root develops on callous tissue in a wound, from scions, buds, on the union, due to environmental conditions and due to inherent stock tendencies;" and later (8) "95 per cent of cases of crown gall are not caused by infection."

Riker and Keitt (12) reported that, in an examination of over 175 trees rejected as crown-gall trees in the nursery, the crown-gall organism was not found. They also stated that malformations resembling certain types of crown gall and hairy root were found on apple trees from grafts made aseptically and grown in steamed soil. They concluded that "of the several working hypotheses which might be advanced to conform with these results, the most promising one appears to be that the malformations dealt with on the rejected nursery trees were not induced by the crown-gall organism." They reported that their experiments indicated that fresh callus on apple graft "is not ordinarily an open infection court for the crown-gall organism."

Waite and Siegler (20) in making recommendations for the control of crown gall in the apple nursery stated that their results were not "in accord with the hypothesis of Riker and Keitt." They concluded that "it is difficult to assume that these malformations are due to any agency other than a pathogenic organism, since they are so definitely prevented by germicides."

Riker and Muncie (14, 15) reported: "The common knot, which has been frequently called gall, appears in most cases to be merely excess callus." They mentioned satisfactory control by mechanical methods.

A summary of an address by Keitt (1) on investigations made by himself and his colleagues stated: "We have two types [of malformations]: (1) Knots formed by true bacterial crown gall, and (2) wound overgrowths." He continued: "One of the striking characteristics of wound overgrowths is root growth on the galls which is seldom present on true bacterial galls." The report on the use of fungicides, including Semesan (an organic mercury compound) as a means of control, concluded: "There were two ways in which chemicals may minimize the growth of galls: By extending the life of the wrapper; by dipping grafts in a strong solution a certain amount of injury to the callus is caused and the tip of the too long scion may be cut off as with a knife."

Melhus (5), in reporting as chairman of the crown-gall committee, Crop Protective Institute, stated: "That we have in the past confused excess callus called hard gall, with true crown gall; and nonpathogenic hairy root with pathogenic hairy root, can not be doubted."

Muncie (6, 7) reported on two types of hairy root on French apple seedlings. The type which he designated the "woolly-knot form, arising from a distinct gall," was considered to be infectious and to have been induced by artificial inoculation of healthy seedlings with *Bacterium tumefaciens*.

Muncie (7) also reported cultures from overgrowths on piece-root apple grafts. This work, as well as that performed simultaneously by Riker and Keitt (13), has been discussed by Siegler (16), who

reported the isolation of an organism, tentatively referred to as the apple strain of *Bacterium tumefaciens*, from the most prevalent type (woolly knots) of malformations found on root-grafted apple trees. The necessity of using the proper host in testing for the infectiousness and the identity of organisms isolated from malformations on apple trees was emphasized. The fact that the woolly-knot type of malformation was identical with the wound overgrowth of Riker and Keitt (13) and Muncie (7) was also noted, but emphasis was placed on the fact that the illustrations of the malformations that these investigators had produced by growing apple grafts in steamed soil did not conform to the type commonly known as woolly knot. Evidence supporting the conclusion that this organism is pathogenic on the aboveground parts of apple as well as on other hosts was produced.

Patel (9) reported on the isolation of 15 nonpathogenic strains of *Pseudomonas* [*Bacterium*] *tumefaciens* from grafted apple trees. Subsequently, Patel (10) reported that the nonpathogenic strains caused no injury on a number of hosts, including apple.

Riker et al. (11) recently reported on "The relation of certain bacteria to the development of roots." They referred to Muncie's work wherein he stated that the woolly-knot malformation is a manifestation of crown-gall infection. They did not note that Muncie's illustrations (7), which show malformations that he concluded were merely wound overgrowths of noninfectious nature, were typical of what Hedgcock (2) had classed as the woolly-knot type of hairy root or crown gall.

The terms "hairy root" and "crown gall" are frequently used interchangeably. It has been customary for pathologists and nurserymen to refer to the woolly-knot type of hairy root as crown gall and not as hairy root. This type of malformation is essentially a knot or gall more or less covered with roots and, as noted previously by Siegler (16), is the type of malformation Riker and Keitt (12) referred to when they reported on the cultural examination of 175 apple trees rejected at the nurseries because of "malformations at the union (supposedly crown gall)," and later, as stated above, referred to by these writers as "wound overgrowth of nonbacterial origin."

Riker et al. (11) in a recent publication stated that in their previous investigations—

were isolated organisms which were similar to *Bact. tumefaciens* in certain routine diagnostic characters, but which failed to induce positive evidences of infection when inoculated through punctures into stems of tomato, tobacco, Pelargonium or apple.

Recently these organisms were inoculated on the underground parts of 1 or 2 year old grafted apple trees of the Wealthy variety, and the writers stated that "at the end of the growing season no typical crown gall or hairy root was observed." They found, however, certain cases where small enlargements or sparse root development occurred. In another series of experiments in which the same inoculum was used, they reported the occurrence of fleshy roots in the places of inoculation. These fleshy roots "occurred singly or in groups and usually arose from slight enlargements." A period of approximately six weeks after inoculation was allowed to intervene before the readings on this experiment were made. In another series of experiments, young shoots of apples were inoculated with one of these strains which

previously had failed to infect when introduced through punctures into stems of the apple and other hosts, and in this series of experiments enlargements and excessive root development occurred. These workers discussed the differences in relation to both physiology and pathogenicity of this organism with what they designate as typical *Bacterium tumefaciens* and concluded that it is probably of specific rank. In referring to this so-called root-stimulating organism, they questioned whether it was harmful or beneficial to the plant, and they noted a possibility that it might be adapted to use in plant culture to facilitate root development.

EXPERIMENTS

M. B. Waite, in 1920, informed the writer that he had secured considerable control of crown gall of apple as early as 1909 by disinfecting with a solution of formaldehyde the scions and stock previous to grafting. In 1921 these experiments of Waite were repeated, and the results of his investigations were confirmed. In 1923 an organic-mercury compound was used in connection with disinfecting the scions and stocks before grafting. Excellent control was secured. These facts apparently supported the conclusion that the woolly-knot type of malformation is pathogenic in its nature. This conclusion was further supported by the fact that the writer had observed that galls on grafts treated aseptically up to the time of planting were, as a rule, smaller than those on grafts not so treated. It appeared probable that the smaller size of the galls on grafts which were kept under aseptic conditions for a brief period at least was due to the fact that infections occurred relatively late on them.

In 1925 a bacterial organism was isolated from malformations identical with the woolly-knot type of hairy root or crown gall, as illustrated by Hedgecock (2), and occurring at the graft union of apple trees. Inoculations with this organism caused malformations on Paris daisy stems. Before the isolations were made, macerations from the tissues of malformations on grafted apple trees had been inoculated into the daisy and slight reactions noted.

In a number of preliminary experiments, this organism, which in lieu of a better designation will be referred to herein as the apple strain of *Bacterium tumefaciens*, was inoculated on the roots of apple. Because the control trees frequently produced enlargements similar to those on the inoculated trees, the technic of these preliminary experiments was not considered satisfactory.

In 1927 a series of inoculations was made on 1-year-old seedling French crab-apple stocks. In addition to the apple organism a strain obtained from a peach gall was used. Small horizontal slits, approximately one-fourth inch in width, were made on the roots by means of a scalpel. The details of this experiment are given in Table 1. It should be noted that inoculations with the peach strain resulted in a higher percentage of infection than those with the apple strain; the controls were almost free from infection.

TABLE 1.—Inoculations of 1-year-old French crab-apple seedlings with strains of *Bacterium tumefaciens* in 1927

Experiment No.	Source of inoculum	Seedlings inoculated and planted	Seedlings surviving	Stand	Slits and root tips inoculated on surviving seedlings	Results of inoculations	
		Number	Number		Number	Slits and root tips doubtfully galled	Slits and root tips galled
			Per cent			Number	Per cent
58-A	Apple strain (455-9-A-9)	25	14	56	84	7	12
58-B	Apple strain (461-A-9)	25	18	72	108	5	14
58-C	Apple strain (474-B-1)	25	23	92	138	4	18
58-D	Apple strain (486-1)	25	24	96	144	3	7
58-E	Apple strain (487-6)	25	19	76	114	12	13
Total	Apple strains	125	98	78	588	31	64
58-F	Peach strain	25	17	68	102	9	30
58-G (control)	Sterile water	25	18	72	108	11	2
58-H (control)	Sterile knife	25	11	44	66	7	2
Total	None	50	29	58	174	18	4



FIGURE 1.—Typical malformations produced on apple-seedling roots: A and B, inoculated with the apple organism; C and D, inoculated with the peach organism. Only the apple organism was recovered from all four specimens. Slightly reduced

The type of malformation, however, was identical, regardless of whether the seedlings were inoculated with the apple or the peach strain or were uninoculated. It was, therefore, not surprising to isolate only the apple strain from typical specimens in each of these three lots. Figure 1, A and B, shows typical malformations obtained on the seedlings inoculated with the apple organism, and Figure 1, C and D, typical malformations obtained on the seedlings inoculated with the peach organism. The malformations on the controls were identical with these. Isolations were made from the galls illustrated in Figure 1 and from malformations on the controls, and the isolated organism, typical of the apple strain, was tested on various hosts and its identity proved.

The explanation offered to account for the high percentage of malformations obtained on the comparatively few seedlings inoculated with the peach organism is that natural infection occurred. This explanation is based on the facts that the apple strain, and not the original inoculum, the peach strain, was isolated from these galls, and that the galls were typical of those produced by infection with the apple strain and were not the smooth, rounded galls obtained by inoculation with the peach organism or with other strains obtained from similar apple galls. Figure 2 illustrates the type of gall obtained by an artificial inoculation with the peach organism which is probably identical with the daisy and the peach strains of Smith et al. (17).



FIGURE 2.—Type of malformation on apple-seedling root resulting from inoculation with the peach strain. Natural size

It is practically identical with the type of gall that Riker and Keitt (13, *pl.* 38) and Muncie (7, *pl.* 2, A) considered as the only pathogenic type. Further consideration of this subject will be found under "Discussion," but it should be noted that the explanation given above loses some of its plausibility when the fact that the controls were practically clean is taken into consideration. As the results of this experiment did not afford an interpretation that might be considered conclusive, they are given for information only, and not as confirmatory evidence of the pathogenicity of these two strains. In 1927 apple grafts were inoculated by being soaked in heavy suspensions of the organisms in sterile water. This experiment was undertaken to secure evidence concerning the pathogenicity of the apple and the peach organisms and the stage at which apple grafts are most susceptible to infection. The fact that a surface disinfection of scions and roots before grafting had given a considerable degree of control indicated that infection may occur quite generally before the grafts are fully callused. This point was mentioned as a probability by Hedgcock (2) and was supported by an experiment wherein grafts were disinfected at definite stages in their development. (Table 2.) The same disinfectant, a 0.25 per cent solution of hydroxymercurichlorophenol, has been used throughout these experiments. Disinfecting the scions and stock gave practically the same control as disinfecting the freshly made grafts, but disinfecting the grafts after they had callused resulted in a high percentage of galled trees.

TABLE 2.—*Results of disinfecting Jonathan apple grafts at different periods*

[100 grafts treated at each period]

Treatment	Resulting trees			
	Number dug	Number smooth	Number galled	Per cent galled
Scions and stocks disinfected before grafting.....	69	64	5	7
Grafts disinfected before callusing.....	68	61	7	10
Grafts disinfected after callusing.....	71	46	25	35
None.....	56	29	27	48

Accordingly, grafts aseptically made were immersed for definite periods in various inocula, either at the time they were made; that is, before callusing, or just previous to planting, after they had callused. The details of this experiment are given in Table 3. Although the percentage of infection obtained by inoculating the grafts at the time of grafting was extremely large as compared with that obtained by inoculating the grafts after they had callused, and although the control grafts and the grafts inoculated with the peach organism remained practically free of infection, the evidence was considered as of supplemental value only, because the numbers used were rather small and the stand obtained was poor. A definite indication, however, was given to strengthen the hypothesis that the freshly made graft was an open court for infection for at least one strain of the crown-gall organism.

In Table 3 are also included data obtained by making two horizontal slits on the scion part and two on the root part of each graft. In these cases the grafts were allowed to callus, the slits were made, and the grafts were then immersed in the inocula as indicated. The result of this part of the experiment was considered to furnish evidence concerning the pathogenicity of the apple strain. Only indirectly did it support the main thesis that the woolly-knot type of crown gall at the graft union is pathogenic in nature and is not merely a mechanical wound overgrowth. In contrast to the result obtained on apple seedlings reported in Table 1, the control slits and the slits inoculated with the peach strain remained free of infection. This supports the explanation of natural infection given to account for the results obtained in the previous experiment.

The experiments of 1927 were repeated with slight modifications in 1928. One-year-old apple seedlings as well as grafts of different varieties on various stocks were used. The seedling apple trees were originally obtained from seed of several open-pollinated varieties, and the grafts were made with stocks which were clons of seedlings obtained from several varieties, furnished by Guy E. Yerkes, of the Bureau of Plant Industry. This, of course, made for uniformity in the experiment. In the 1928 experiments the inoculated and control seedlings were allowed to callus for 22 days before they were planted, in order to keep the wounds free from contaminants after planting. The seedlings were inoculated by making small horizontal slits in them and then soaking them in suspensions of the inoculum. The results of these inoculations are given in Table 4.

TABLE 3—Inoculations of apple grafts with strains of *bacterium tumefaciens* in 1927

Experiment No.	Host	Source of inoculum	Time of applying inoculum with reference to callusing	(Grafts planted)	(Grafts surviving)	Stand	Results of inoculation										Scion slits inoculated		Scion slits galled		Root slits inoculated		Root slits galled	
							At graft union		At root tip															
							Doubt-fully galled	Galled	Doubt-fully galled	Galled	Num-ber	Per cent	Num-ber	Per cent	Num-ber	Per cent	Num-ber	Per cent	Num-ber	Per cent	Num-ber	Per cent	Num-ber	Per cent
38-A	Buckskin on French crab.	Apple strain (455-9-A-9)	Before	60	4	6.6	0	3	75.0	0	4	100												
38-B	do.	Apple strain (461-A-9)	do.	78	14	17.9	0	14	100	0	14	100												
38-C	do.	Apple strain (474-B-1)	do.	56	16	28.5	0	14	87.5	0	13	81.2												
Total	do.	Apple strains	do.	194	34	17.5	0	31	91.1	0	31	91.1												
38-D	do.	Peach strain	do.	65	26	40.0	1	0	0	1	0	0												
38-E (control)	do.	Sterile water	do.	45	33	73.3	0	0	0	0	3	9.1												
38-F	do.	Apple strain (455-A-9)	After	45	23	52.2	1	1	4.3	2	10	43.5												
38-G	do.	Apple strain (461-A-9)	do.	45	26	57.7	1	4	15.4	0	10	38.5												
38-H	do.	Apple strain (474-B-1)	do.	45	33	73.3	1	3	9.1	2	8	24.2												
Total	do.	Apple strains	do.	135	82	60.7	3	11	13.4	4	28	34.1												
38-I	do.	Peach strain	do.	45	35	77.7	2	0	0	0	0	0												
7-A	Givens on French crab.	Apple strain (455-9-A-9)	do.	35	18	51.4	0	4	22.2	0	2	11.1	36	9	25.0	36.1	13	36.1						
7-B	do.	Apple strain (461-A-9)	do.	35	24	68.5	0	4	16.6	0	0	0	48	13	27.1	48	15	31.3						
7-C	do.	Apple strain (474-B-1)	do.	35	19	54.2	0	3	15.7	0	0	0	38	4	10.5	38	11	28.9						
7-D	do.	Apple strain (486-1)	do.	35	23	65.7	0	1	4.3	0	0	0	46	17	36.9	46	21	45.6						
7-E	do.	Apple strain (487-6)	do.	35	27	77.1	0	1	3.7	0	0	0	54	13	24.1	54	22	40.7						
Total	do.	Apple strains	do.	175	111	63.4	0	13	11.7	0	2	1.8	222	56	25.2	222	82	36.9						
7-F	do.	Peach strain	do.	20	11	55	0	0	0	0	0	0	22	0	0	22	0	0						
7-G (control)	do.	Sterile water	do.	20	13	65	0	0	0	0	0	0	26	0	0	26	0	0						

TABLE 4.—Inoculations of 1-year-old apple seedlings with the apple strain of *Bacterium tumefaciens* in 1928

Experiment No.	Seedling host	Seedlings inoculated and planted	Seedlings surviving	Stand	Slits and root tips inoculated on surviving seedlings	Results of inoculation					
						Slits galled			Root tips doubtfully galled		Root tips galled
						Number	Percent	Number	Number	Percent	
136-A	Delicious	54	53	98.1	159	56	52.8	2	1	1.8	
136-A (control)	do	54	49	90.7	147	0	0	2	0	0	
136-B	McIntosh	50	45	90.0	135	40	44.4	4	13	28.8	
136-B (control)	do	48	38	79.1	114	0	0	4	0	0	
136-C	Tolman	100	88	88.0	264	72	40.9	0	70	80.0	
136-C (control)	do	100	88	88.0	264	0	0	8	0	0	
136-D	Northern Spy	54	45	83.3	135	50	55.5	6	14	31.1	
136-D (control)	do	50	43	86.0	129	0	0	4	0	0	
136-E	Yellow Siberian	55	54	98.2	162	102	94.4	0	30	55.6	
136-E (control)	do	50	47	94.0	141	0	0	5	0	0	
Total (inoculations)		313	285	91.1	855	320	56.1	12	128	44.9	
Total (controls)		302	265	88.1	795	0	0	23	0	0	

It is believed that the numbers used, the stand that was obtained, the uniformity of the stock, the high percentage of galls resulting on the inoculated trees, and the freedom of the controls from infection are all factors that should be considered as justifying the drawing of definite conclusions from the results of this experiment. In this experiment as well as in subsequent ones the seedlings were disinfected with an organic-mercury solution before they were used. The disinfecting solution was always washed off with distilled water. The fact that the seedlings in the controls were also disinfected and that the wounds were partially closed by callus before planting may account for their rather unusual freedom from natural infection. It is noted that of the 855 slits and root tips where the organism was afforded opportunity to infect, 448, or 52.4 per cent, became infected. On the 795 control slits and root tips no infection was found. The groups of inoculated and uninoculated Yellow Siberian seedlings are illustrated in Figures 3 and 4, respectively. Typical malformations resulting from inoculation with the apple organism and the controls free of galls are shown in Figure 5, A and B, and Figure 5, C and D, respectively. Reisolations from these galls, typical of those always resulting from infection with the apple organism, were not attempted in this case.

The series of experiments on apple grafts in 1928 gave results entirely in accord with those obtained on the apple seedlings in 1928. (Table 5.) The preponderance of infection resulting from subjecting the newly made graft to the inoculum previous to callus formation as compared with the infection of those grafts which were subjected to the inoculum after they were allowed to callus is evident. Again, as in the experiments of the preceding year the grafts inoculated with the peach organism and the control grafts remained free of galls. The total freedom of these control grafts from natural infection may be due to the disinfection of the scions and stock or to the absence of the inoculum in the soil in which the grafts were planted.

TABLE 5.—Inoculations of apple grafts with strains of *Bacterium tumefaciens* in 1928

Experiment No.	Host	Source of inoculum	Time of applying inoculum with reference to callusing	Results of inoculation												Scion slits inoculated	Scion slits galled	Root slits inoculated	Root slits galled	
				Grafts planted		Grafts surviving		Stand	At graft union				At root tip							
				Num-ber	Per cent	Num-ber	Per cent		Galled		Doubt-fully galled		Galled		Doubt-fully galled					
									Num-ber	Per cent	Num-ber	Per cent	Num-ber	Per cent	Num-ber					Per cent
127-B	Northwestern Greening on French crab.	Apple strain (486-1)	After	94	70	74.5	4	3	4.3	1	0	0	140	42	140	63	45			
127-C	do.	Peach strain.	do.	94	63	67.0	2	0	0	0	0	0	126	0	126	0	0			
127-A (control)	do.	Sterile water.	do.	94	67	71.3	0	0	0	0	0	0	134	0	134	0	0			
128-A	Yellow Transparent on Yellow Siberian.	Apple strain (486-1)	Before	50	28	56.0	0	26	92.9	3	3	10.7								
128-B	do.	Peach strain.	do.	50	41	82.0	1	0	0	0	0	0								
128-D	do.	Apple strain (486-1)	After	50	45	90.0	0	4	8.9	2	2	8								
128-E	do.	Peach strain.	do.	50	38	76.0	4	0	0	0	0	0								
128-C (control)	do.	Sterile water.	do.	50	38	76.0	0	0	0	0	0	0								
129-A	Winter Banana on French crab.	Apple strain (486-1)	Before	100	67	67.0	0	49	73.1	9	30	44.8								
129-B	do.	do.	do.	100	73	73.0	8	27	37.0	2	13	17.8	146	1	146	5	3.5			
129-E	do.	do.	After	100	72	72.0	2	3	4.2	1	0	0								
129-F	do.	do.	do.	100	62	62.0	0	7	11.3	2	2	3.2	124	43	124	36	29			
130-C (control)	do.	Sterile water.	Before	100	57	57.0	3	0	0	0	0	0								
130-B	Yellow Transparent on Florence crab.	Apple strain (486-1)	do.	57	35	61.4	2	28	80.0	3	24	68.5								
130-C	do.	Peach strain.	do.	57	38	66.7	5	0	0	0	0	0								
130-A (control)	do.	Sterile water.	do.	57	41	71.9	5	0	0	0	0	0								
131-B	Delicious on French crab.	Apple strain (486-1)	do.	75	21	28.0	1	17	81.0	0	9	42.8								
131-C	do.	Peach strain.	do.	75	30	40.0	0	0	0	0	1	3.3								
131-A (control)	do.	Sterile water.	do.	75	36	48.0	0	0	0	0	0	0								
132-B	Yellow Transparent on French crab.	Apple strain (486-1)	After	50	23	46.0	3	9	39.1	4	3	13.0								
132-A (control)	do.	Sterile water.	do.	50	38	76.0	6	0	0	0	0	0								
Total (5 lots)		Apple strain (486-1)	Before	382	224	58.6	11	147	65.6	17	79	35.3								
Total (5 lots)		do.	After	394	272	69.0	9	26	9.6	10	13	4.8								
Total (6 lots, control)		Sterile water.		426	277	65.0	14	0	0	0	0	0								

Galls typical of those appearing on the grafts inoculated with the apple strain are shown in Figure 6, A-C. The organism was reisolated from the specimen in Figure 6, B, which showed unusual freedom from root developments on its surface. Attention is directed to the data given in Table 5 under the heading "Results of inoculation at graft union, galled" for the varieties Yellow Transparent on Yellow Siberian seedlings and Winter Banana on French crab seedlings, where



FIGURE 3.—Entire lot of 54 Yellow Siberian seedlings, showing numerous woolly-knot malformations resulting from inoculations with the apple organism in experiment No. 136-E. (Compare with Figure 4.) Greatly reduced

a comparison of grafts inoculated both before and after callusing may be found.

When attempts have been made to secure galled trees at certain periods of the year, especially in midsummer, there has always been difficulty in finding abundant specimens of galls on grafts planted the preceding spring. This fact led to the hypothesis, that the high nutritional condition of the host at the point of infection may be a

factor of greater importance in the degree of pathogenicity exhibited than the state of rapid growth of the tissues at that point. The conception that rapidly growing tissues favor infection has been generally accepted. In order to test this hypothesis the following experiment was performed. Apple roots were inoculated by means of small horizontal slits, approximately one-fourth inch in width and one-eighth inch in depth, at two periods in midsummer. The inocu-



FIGURE 4.-- Entire lot of 47 uninoculated Yellow Siberian seedlings, free of malformations. (Compare with Figure 3.) Greatly reduced

lations were made by inserting the inoculum from an agar slant of the apple organism into a slit made by means of a scalpel on the scion wood from one-fourth to 2 inches above the union, on grafts which had been planted the current spring. The details and results of these experiments are given in Table 6. Although the number of grafts used in this experiment was not large, it is felt that the results are so definite that, when coupled with the results of other experiments reported here, there is presented proof that the so-called apple strain

of crown gall is responsible for the type of woolly-knot galls found on grafted apple trees. It should be noted, however, that in this experiment inoculations were made on the scion wood and not on the graft union itself.

Galls resulting from inoculations made on August 20 were as large as those obtained from inoculations made on July 19. The roots on



FIGURE 5.--A and B, Woolly-knot galls on apple-seedling roots resulting from inoculations from the apple organism. C and D, Control seedlings, free of malformations. All natural size

the galls of trees inoculated on July 19 were mostly of the fibrous type, whereas the roots on the galls of the trees inoculated in August were invariably of the fleshy type. Typical illustrations of galls from these lots are shown in Figures 7, A and B, and 8, A to C, respectively. The organism was reisolated from the specimens illustrated in Figures 7, A and B, and 8, B and C.

TABLE 6.—Results obtained by inoculating Yellow Transparent scion tissue adjacent to the graft union with the apple strain of *Bacterium tumefaciens* on July 19 and August 20, 1928

Experiment No.	Date of inoculation	Grafts inoculated	Grafts surviving	Slits inoculated on grafts	Slits galled	
		Number	Number	Number	Number	Per cent
163.....	July 19..	15	13	13	13	100
163 (control).....	do.....	15	14	14	0	0
175.....	Aug. 20..	50	50	50	50	100
175 (control).....	do.....	25	25	25	0	0



FIGURE 6.—A-C, Types of malformations on Winter Banana apple grafts resulting from inoculations with the apple organism. Natural size

In controlling malformations on the graft union, Hedgecock emphasized the importance of making well-fitted grafts. In 1921 the writer initiated experiments for the purpose of testing the effect on malformations of the use of well-matched and poorly matched grafts. In a general way it may be stated that the use of well-matched grafts lessened to some extent the number of malformations. Quite frequently, however, the well-matched grafts did not yield a larger percentage of clean trees than the poorly matched ones. Riker and Muncie (14) and other workers in this field have emphasized the importance of well-matched grafts as a means of controlling malformations. The writer, therefore, believing that perhaps there had not been sufficient contrast between well-matched and poorly fitted grafts in his previous experiments, repeated this experiment, making certain that the well-matched grafts were as perfect as it was possible

for an expert grafter to make them, and having the poorly fitted grafts made with the lower end of the scion blunt in some cases and pointed and extending over the cut surface of the seedling in others. The results of this experiment are given in Table 7.

TABLE 7.—Results from comparative plantings of untreated poorly matched and well-matched grafts of Duchess apple

[100 of each kind of graft]

Description of graft	Resulting trees			
	Total	Number smooth	Number galled	Per cent galled
Poorly matched:				
Lower end of scion blunt.....	67	1	66	98.5
Lower end of scion pointed.....	78	12	66	84.6
Well matched.....	71	8	63	88.7

It will be seen that an exceedingly large number of malformations, practically all on the graft union, resulted on all these grafts; and since 88.7 per cent appeared on the perfectly matched grafts, it is evident that the reliance on well-matched grafts alone as a control of malformations can be overemphasized.

DISCUSSION

As previously stated, the term "woolly knot" has been quite generally recognized as referring to a type of malformation characterized by a swelling more or less covered with fibrous or fleshy roots. Whether one designates this type of malformation as crown gall or as a form of hairy root is of small moment, as the terms have generally been used interchangeably. To prevent any possible confusion, however, the writer points to Figures 1 and 6 as illustrations of what he considers typical woolly-knot malformations. Malformations typical of what the writer terms "woolly knot" are found in illustrations designated as wound overgrowths by Riker and Keitt (13, *pl. 40, C*) and by Muncie (7, *pl. 2, B*). There is a tendency, perhaps unfortunate, to designate malformations exhibited on seedling roots as hairy root, while the same type of malformation on grafted trees would be generally termed "woolly knot." Whether this type of malformation is found on a 1-year-old seedling or on a 1-year-old grafted tree should not affect its status. Smith et al. (17, *pl. 18, fig. 1*) and Hedgecock (2, *pl. 6, fig. 5*) have also illustrated what the writer considers typical woolly knots. The malformations obtained by Smith et al. were secured by artificial inoculation of apple seedlings with what they termed the apple organism and were designated by them as apple hairy root. This type of malformation apparently is identical with those illustrated in Figure 1 of the present paper which resulted from inoculations with the apple organism.

The type of gall that Riker and Keitt (13) considered true bacterial gall is shown in their Plate 38, A-C. Muncie (7, *pl. 2, A*) also illustrated what he considered as typical galls, from which he isolated *Bacterium tumefaciens*. A very similar type of gall is shown

in Figure 2 of the present paper. This gall resulted from an inoculation on apple roots with the peach organism of Smith, isolated from a peach gall. It is presumably the only type of malformation on grafted apple trees that Riker and Keitt (13) and Muncie (7) recognize as being pathogenic in nature, and it is caused by the strain that is apparently identical with the peach and daisy strains of Smith et al. (17). Undoubtedly other strains may cause similar types of galls. It is seen that the dissimilarity between the rather smooth type of gall caused by the peach strain or similar strains and the woolly-knot type resulting from infections with what the writer terms the apple strain is quite marked. The distinction



FIGURE 7.—A and B, Typical malformations on the scion wood of Yellow Transparent apple grafts resulting from inoculations with the apple organism. The roots from inoculations made on July 19, 1928, were more fibrous than those from inoculations made on August 20, 1928. (Compare with fig. 8, A-C.) Natural size

between these two types of galls is, of course, necessary for a proper comparison and interpretation of the results of these experiments, especially in so far as they are related to the investigations of others.

The experiments detailed in Table 1 were performed in a manner somewhat similar to one described by Muncie (7) except that Muncie used apple seedlings grown from seed planted the current year in soil that had been steamed, whereas the writer used 1-year-old transplanted seedlings in unsteamed soil. Muncie made inoculations with what was possibly the peach strain or at least a strain which produces similar results, whereas the writer used both apple and peach strains. Inoculations with the peach strain resulted in

9.15 and 29 per cent infection in Muncie's and in these experiments, respectively. However, in the writer's experiments the type of gall obtained was identical with the type that always resulted from inoculations with the apple organism. These galls were decidedly not of the type caused by the peach strain (fig. 2), and moreover when cultured they yielded, as was expected from their appearance, the apple organism. Muncie's experiments indicated that inocu-



FIGURE 8.—A-C, Fleshy roots from malformations secured as a result of inoculations made on August 20, 1928, on scion wood of Yellow Transparent apple grafts. (Compare with fig. 7, A and B.) Natural size

lations with the peach strain resulted in the "woolly-knot form of hairy root." No report of isolations is given, and it is perhaps reasonable to assume that the woolly knots obtained in Muncie's experiments would have yielded in culture the apple organism and not the original organism. On the other hand, it might be assumed that the peach organism was actually present in the galls obtained by the writer and the culturing technic failed to reveal it. This

latter assumption loses force, however, when it is recalled that the galls were typical of those invariably obtained with the apple organism. Still, the fact that the controls in each of these experiments were practically free of infection must be considered. To regard the natural infections on the seedlings inoculated with the peach organism in the writer's experiments as fortuitous might be permissible; to apply the same conception to Muncie's experiments, in steamed soil, is permissible only with qualifications. In view of the results of the subsequent experiments reported here, wherein inoculations on apple grafts with the peach organism practically failed of infection, whereas those with the apple strain resulted in high percentages of infection, it is considered that, regardless of any interpretation of this particular experiment, the results and interpretations of the other experiments are not materially influenced by this one. This experiment is reported not as furnishing evidence but rather as illustrating how diametrically opposed conclusions can be drawn from the similar results of similar experiments.

Riker et al. (11) in a recent reference to Muncie (7), stated that he considered that the "woolly-knot form is a manifestation of crown-gall infection." It seems desirable to direct attention to the fact that Muncie's experiments from which these conclusions were drawn were on apple seedlings and not on apple grafts and that galls on apple grafts, typical of woolly knots, were illustrated by Muncie (7, *pl. 2, B*) as being the type of malformations "from which *Ps. tumefaciens* was not recovered" and hence considered by him non-pathogenic overgrowths, and finally, that it is quite possible, as has been stated, that the malformations which Muncie secured by means of inoculations with the peach strain actually resulted from natural infections of the apple organism and not from the original inoculum. The conclusion drawn by Muncie that "the woolly-knot form is a manifestation of crown-gall infection" when it occurs on apple seedlings but that "the abundant development of fibrous roots from an overgrowth at the union of piece-root grafted trees is not a reliable index of crown-gall infection" may not be tenable.

CAUSE OF THE WOOLLY-KNOT TYPE OF MALFORMATION

The writer (16) has reported on the rather consistent isolation of the apple organism from the woolly-knot type of malformations and on the pathogenicity of this strain of the crown-gall organism on apple shoots as well as on other hosts. The fact that so many isolations were made and reported in previous experiments is the reason why only comparatively few isolations were made in these investigations.

Evidence shown in Table 5, supplemented by that in the other tables, consistently shows that the apple organism is pathogenic on apple roots at the graft union and causes the type of malformation most commonly encountered in the root-grafted apple nursery. It is considered that the experiments have contained sufficiently large numbers, have been repeated often enough, and have yielded results so consistent as to afford conclusive interpretations of the results.

In the recent report of Riker et al. (11) concerning their inoculations on underground parts of apple shoots with strains of bacteria considered to "closely resemble" *Bacterium tumefaciens*, it is noted that

these strains, which previously had failed to cause infection when inoculated into tomato stems, did result in infections, characterized as "enlargements which occurred at the bases of these excessive root developments * * *," when inoculated on the underground shoots of apple. It is quite possible that these so-called root-stimulating organisms of Riker et al. (11) may play a leading rôle in connection with the woolly-knot malformations on apple grafts or seedlings. Regardless of the question of the specific identity of the organisms used by these investigators, the writer considers that their recent investigations furnish strong evidence supporting the pathogenic conception of woolly-knot malformations.

STRAINS OF BACTERIUM TUMEFACIENS

Since a prolonged discussion of the strains of *Bacterium tumefaciens* is not within the scope of this paper, reference is made to Smith et al. (17). Patel (9) noted the occurrence of what he termed nonpathogenic strains of *Bacterium tumefaciens*. Riker et al. (11) reported the isolation of "* * * organisms which were similar to *Bact. tumefaciens* in certain routine diagnostic characters * * *" from "a large majority of the naturally occurring malformations" and noted that this organism is capable of causing a reaction on the underground shoots of apples. It is quite probable that this organism is closely similar to, if not identical with, the one referred to by the writer (16) as the apple strain. The fact that Patel (10), however, was unable to secure infection on the apple with what he termed some "nonpathogenic" strains of *Bact. tumefaciens* does not support this impression. It is further noted that Riker et al. (11) reported that in their previous experiments these organisms "failed to induce positive evidences of infection when inoculated through punctures into stems of * * * apple." The interpretation to be placed on the word "positive" (evidence) is mootable. However, the condition of the host, as well as the technic used, the virulence of the culture, and other factors may account for some discrepancies. Stapp (19) and other European workers also recognized widely divergent strains of the crown-gall organism. As stated previously, the writer (16) considers that there are just as wide divergences in other species of bacteria.

SUSCEPTIBILITY OF NEWLY MADE GRAFTS TO INFECTION

As stated, the condition of the hosts, the technic, the age and virulence of the inoculum, the temperature and moisture relations, and numerous other factors are of especial importance in experiments with the crown-gall organisms.

Hedgcock (2) expressed the opinion that most apple-root grafts become diseased with crown gall apparently at the time of the formation of the callus. Riker and Keitt (13) concluded, as a result of their experiments in which the callused grafts were immersed in the inoculum, that the freshly produced callus is not an open infection court for the crown-gall organism. In view of the fact that the writer had secured a large measure of control of the woolly-knot malformation by disinfecting the scions and stock before grafting, an experiment was planned, as stated previously, to determine at just what time subsequent to grafting the grafts were most subject to infection. Inoculations on grafts were made with the peach and

the apple strains, not only subsequently but also previously to callus formation. This experiment (Table 5) gave in part the same results as the one performed by Riker and Keitt but admitted of a different interpretation. Here the peach strain failed to cause infection, just as did the strain that Riker and Keitt used. The freshly formed callus is not an "infection court" for the peach or similar organisms, as Riker and Keitt (13) rightly concluded, but it is extremely susceptible tissue for the apple organism. The evidence furnished by this experiment indicated that most infections occur during the precallusing or early-callusing period, rather than after the callus has formed. Experiments similarly conducted in the season of 1928 completely confirmed this conclusion and again demonstrated that the apple strain was the cause of the resulting malformations. The illustrations in Figure 6 represent malformations typical of those secured by inoculation with the apple organism at the time of grafting. This type of malformation previously yielded the apple strain in culture quite consistently. The controls were practically clean. It is believed that this evidence, offered as proof that most infections occur previous to callus formation, explains why surface disinfection of the seedling stocks and scions affords some measure of control.

It should be noted, however, that Riker and Keitt (13, p. 772) reported negative results in controlling malformations by means of surface disinfection of stocks and scions. The writer's results are not in conformity with those reported by these investigators, who also dipped the freshly made grafts in antiseptics. Perhaps this discrepancy may be explained in part by reference to the writer's (16) previous statement that he did not consider the malformations illustrated by Riker and Keitt (13, pl. 45) or by Muncie (7, pl. 3, b) as being typical of galls most commonly encountered in the root-graft apple nursery.

WELL-MATCHED GRAFTS AS A MEANS OF CONTROL

The writer's experiments have in the main indicated that careful attention to making well-matched grafts may result in a decrease in the number of malformations, but the data given in Table 7 indicate that, under conditions favorable for natural infection, the importance of well-matched grafts as a means of control can be overemphasized.

GROWTH AND NUTRITIONAL CONDITIONS

In the literature relating to crown-gall inoculations there has been a tendency to give considerable attention to the so-called vigor of the host.

In seeking an explanation as to why woolly-knot malformations were not readily found until the fall of the year on grafts planted the current year, and in view of the fact that large galls are found on very small as well as on large yearling trees the thought occurred that perhaps the question of nutrition at the point of infection might be more important than that of vigor or rapidity of growth. The experiments detailed in Table 6 were performed in part to test this hypothesis. Since the malformations on the grafts inoculated August 20 were equally as large as those obtained on the grafts inoculated on July 19, one month earlier, there is an indication that the increased supply of elaborated food materials at the point of inoculation in

late August may be a very important factor in securing infection and in influencing the size of the gall. Although numerous other factors necessarily should be considered in this connection, additional experiments which are so preliminary in nature that it is not deemed advisable to report them here in detail afford evidence to support this hypothesis. In brief, seedling apple trees of the current season's growth have been inoculated with the apple strain of the crown-gall organism by means of needle punctures at a point immediately above the axillary bud. The area immediately below the leaf petiole has been girdled by means of a sharp scalpel in some cases; in other cases the analogous areas have not been girdled. From the results of one season's experiments only, there is evidence that infections have been secured more readily in those areas that were girdled below the leaf petiole.

CONCLUSIONS

Because of the nature of the subject matter in the "Discussion," a recapitulation of the salient points produced by these experiments may be helpful, especially in correlating the interpretations of the results of these experiments with those obtained by other investigators.

The primary purpose of the studies reported in this paper has been to discover the cause of malformations known as woolly knots prevalent on root-grafted apple trees. The question raised as to the cause of this type of malformation by Riker and Keitt (12, 13), by Melhus (4), and by Muncie (7), and the conclusion of these writers that the problem of malformations on apple grafts was one dealing primarily with nonbacterial wound overgrowths, made it advisable not only to perform experiments on the aboveground and underground tissues of the apple but to give special attention to the graft union itself. At this point of contact between the seedling root and scion are found the majority of the malformations. The bacterial organism, previously reported by Siegler (16) as probably being identical with the apple strain of Smith and others (17), was isolated rather consistently from the malformations in question and was found to exhibit a degree of parasitism on apple shoots and other hosts. A limited number of inoculations with another organism, apparently the peach strain of Smith et al., were also made. This organism causes a comparatively smooth, rounded type of malformation on apple roots and apparently is the only type of gall on root-grafted apple trees considered by Riker and Keitt (13) and Muncie (7) as pathogenic. Although Muncie (7) credited a certain amount of woolly-knot hairy root with being infectious on apple seedlings, this conclusion is subject to several interpretations which have been discussed. Furthermore, Riker and Keitt (13) reported: "In cases in which roots have been observed in association with a crown gall they have been found to occur in connection with wound overgrowth that was adjacent to the gall." Thus they concluded that the hairy roots were in fact not a manifestation of infection, and at that time interpreted Muncie's results to be in conformity with their conception that the hairy roots arise from wound overgrowths. Recently, however, Riker et al. (11) have given a different interpretation of Muncie's results and now apparently accept his conclusion, referring to apple seedlings, that the "woolly-knot form is a manifestation of

crown-gall infection." Regardless of the above interpretations and those discussed previously, the cause of the woolly-knot malformations on the graft union has been the chief concern of these experiments.

In order to avoid confusion of issues, it is believed that it is essential to recognize the practical synonymy of the terms "crown gall" and "hairy root" and the identity of the distinct types of galls produced by the peach or closely related strains of the crown-gall organism and by the so-called apple strain. Despite differences between these so-called strains, it is a matter of judgment whether these strains should be considered as worthy of specific rank.

As Riker and Keitt (13) noted, Smith et al. (17) performed only a limited number of experiments on the apple and did not produce evidence sufficient to reach a conclusion concerning the identity of the woolly-knot malformations. In the inoculation experiments of Smith et al. (17) with the apple organism the checks, when used, became infected all too frequently, and no inoculations were made on the graft union; with the peach organism, inoculations on the roots were practically failures. On the other hand, while Smith et al. (17) did not offer conclusive proof in this case, their experiments furnished certain leads which should not be disregarded.

The assumption that the organism referred to in these experiments as the apple strain is identical with the apple strain of *Bacterium tumefaciens* of Smith et al. (17) appears warranted. It is probable that other workers have also isolated this organism but have regarded it as a nonpathogenic strain of *Bact. tumefaciens*. Recently Riker et al. (11) reported the results of inoculations on underground stems of apple with what was probably an organism previously regarded as a nonpathogenic strain of *Bact. tumefaciens*. A root-stimulating effect was noted. These latest experiments of Riker et al. (11) are in agreement with some of the experiments reported here and are thought to confirm the evidence that woolly-knot malformations are caused by bacteria, regardless of the specific identity of the organism.

The fact that the freshly made graft is susceptible at the union to infection with the apple organism is apparently proved by the evidence furnished by these experiments. That the graft union after callus has formed is an infection court to a limited extent for the apple organism is also indicated by the evidence herein shown. The conclusion of Riker and Keitt (13) that the graft union is not an open court for infection for the crown-gall organism holds true for certain strains only but not for the apple strain, which was not reported as being used by these investigators.

The evidence that most of the infections occur during the time the grafts are callusing supports the opinion expressed by Hedcock (2). It also offers a reasonable explanation for the control of malformations when the scions and stock are disinfected previous to grafting.

There is some evidence suggesting that the amount of nutrition at the point of inoculation is more important than a condition of rapidly growing tissue, in the amount and kind of infection secured. If this is correct, an explanation as to why the freshly made graft is especially susceptible to infection with the apple organism is offered. The seedling stock is, of course, comparatively rich in food reserves at the time of grafting.

There was a marked difference in the character of the roots springing from malformations secured as a result of inoculations made in

July and August, respectively. The excessive fleshiness of the roots resulting from inoculations in late summer may be due, among other causes, to a certain degree of immaturity, to a mechanical action, or even to a physiological action whereby only the downward passage of food material is permitted or is active. These are questions not especially germane to the end results of these experiments.

The facts that woolly-knot malformations were produced on graft unions by inoculations with the apple strain of *Bacterium tumefaciens* in a large series of experiments and with the use of a number of varieties, that these woolly knots consistently yield this organism in cultures the validity of which have been proved by reinoculations, and that the control grafts in each of these series have remained practically free of malformations, are considered proof sufficient to warrant the conclusion that this type of malformation is pathogenic and is caused by a bacterial organism which can be tentatively referred to as the apple strain of the crown-gall organism. These conclusions are supported by results obtained on apple seedlings.

SUMMARY

In this paper are reported experiments in which apple grafts and seedlings have been inoculated with an organism tentatively referred to as the apple strain of the crown-gall organism (*Bacterium tumefaciens*). This is probably the same organism that Smith et al. isolated from malformations on apple roots, and because of the wide divergence in strains of the crown-gall organism it is deemed advisable to continue the designation of this organism as the apple strain. This so-called apple strain causes the woolly-knot type of crown gall or hairy root prevalent on root-grafted apple trees.

It is probable that this apple strain is identical or at least closely related to the so-called nonpathogenic strains of *Bacterium tumefaciens* of other workers, since those strains have produced similar results when inoculated on the below-ground parts of apple shoots.

When apple grafts were immersed in an inoculum of the apple organism previous to callus formation, abundant infection occurred; when immersed in the inoculum after callus had formed, infection was not so abundant. One other strain of the crown-gall organism failed to cause infection when inoculated at these periods.

The reason why surface disinfection of scions and stock previous to grafting affords some measure of control may be due to the aseptic condition of the grafts at a time when they are susceptible to infection.

Limited experiments indicate that the nutritional condition at the point of inoculation may be a factor of greater importance than the condition of rapid growth, in the amount and kind of infection secured.

The woolly-knot type of crown gall on apple grafts caused by the apple organism is quite distinct from the comparatively smooth type of gall with slight or no root developments caused by certain other strains of the crown-gall organisms.

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THE MEAN AND VARIABILITY AS AFFECTED BY CONTINUOUS SELECTION FOR COMPOSITION IN CORN¹

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INTRODUCTION

The effectiveness of selection in cross-fertilized crops such as maize may be explained upon the following principles: (1) There are heritable variations existing among the different individuals of the population at the beginning; (2) there is a gradual elimination of the undesirable type because the selected types consist of more desirable and fewer undesirable individuals in each generation; (3) desirable mutations which may occur are retained and caused to combine with the desirable factors present; and (4) recombination of the desirable factors produces more desirable types.

Selection for a given type not only tends to bring the population to that type but is expected to decrease the variability. This decrease in variability of the population is brought about by a reduction in the percentage of heterozygous individuals. After the population becomes homozygous for the selected character, no further reduction in variability through selection can be expected. The variability that still remains is attributed to environment, upon which selection has no influence.

It is the purpose of this paper to present the effect of 28 years of continuous selection for composition in maize upon the mean and the variability of the selected character.

MATERIAL

In 1896 a series of breeding experiments was begun at the Illinois Agricultural Experiment Station to determine whether the chemical composition of corn could be influenced by selection (8).³ One hundred and sixty-three ears of a variety known as Burr's White were used as the foundation stock, from which selections were made in four different directions, namely, for high oil, low oil, high protein, and low protein.

These four strains were carried on in the same way. In the high protein, for example, the 24 ears highest in protein were selected for seed and planted in an isolated plot, each ear in a separate row. These rows were harvested separately and the seed for the next crop selected from the ears which were found to be highest in protein. Nine years later the system was modified somewhat in an attempt to prevent loss of vigor by inbreeding. Alternate rows were detas-

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² The writer wishes to thank Dr. L. H. Smith for suggesting the problem. To Dr. C. M. Woodworth, under whose direction these studies were made, the writer expresses sincere thanks for suggestions in analyzing the data and for valuable assistance in the preparation of this paper.

³ Reference is made by number (italic) to "Literature cited." p. 475.

seled and seed was selected only from the highest yielding detasseled rows. In 1921 this system was again modified to reduce the amount of inbreeding. Two seed ears were taken from each of the detasseled rows regardless of yield. The high-oil, low-oil, and low-protein tests were similarly conducted, selection being made each year of ears highest in oil, lowest in oil, and lowest in protein, respectively.

The analytical methods employed have been described in detail in Illinois Agricultural Experiment Station Bulletins 43 and 53 (6, 7).

EFFECT OF SELECTION FOR PROTEIN CONTENT

The effect of selection for protein content in the corn grain is shown in Figure 1. The average protein content which was 10.92 in

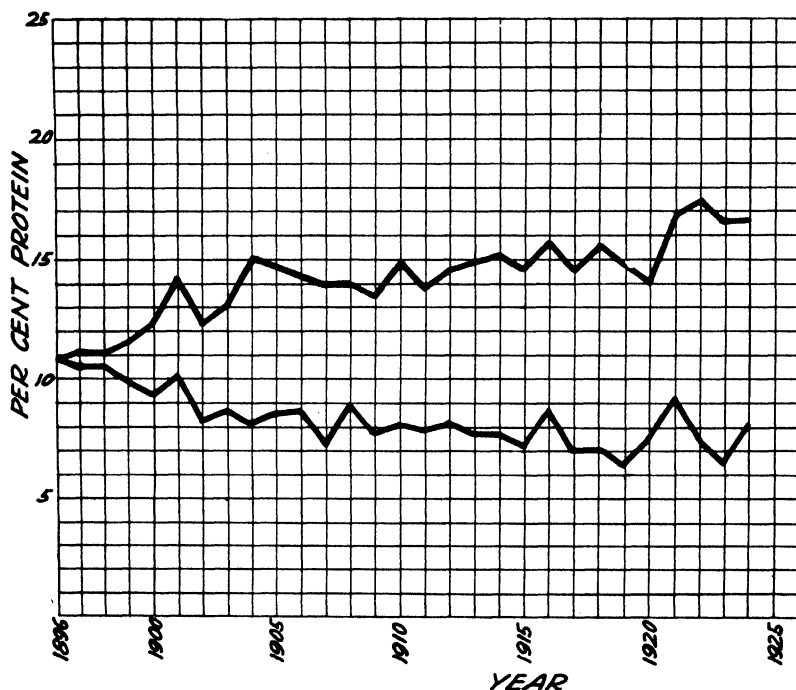


FIGURE 1.—Progress of high-protein and low-protein corn breeding

1896 had in 1924 been increased to 16.60 per cent in the high-protein strain and had been decreased to 8.38 per cent in low-protein strain. This is a difference of 8.22 per cent. When measured by the best fitting straight lines, the difference is 9.49 per cent. When compared with the original variety from which the strains were selected, the proportional increase is 50.01 per cent and the proportional decrease is 23.26 per cent.

EFFECT OF SELECTION FOR OIL CONTENT

From an average of 4.70 per cent in 1896, the oil content had been increased to 9.86 in the high-oil strain and decreased to 1.51 per cent

in the low-oil strain in 1924. This is a difference of 8.35 per cent. When measured by the best fitting straight lines, the difference is 8.85 per cent. Relatively, selection has been much more effective in bringing about a change in oil content than in protein content. When compared with the original variety, the proportional increase in oil is 109.79 per cent and the proportional decrease is 67.87 per cent. (Fig. 2.)

It is very probable that a more rapid shift in type with respect to both the oil and protein would have been obtained if only the ears

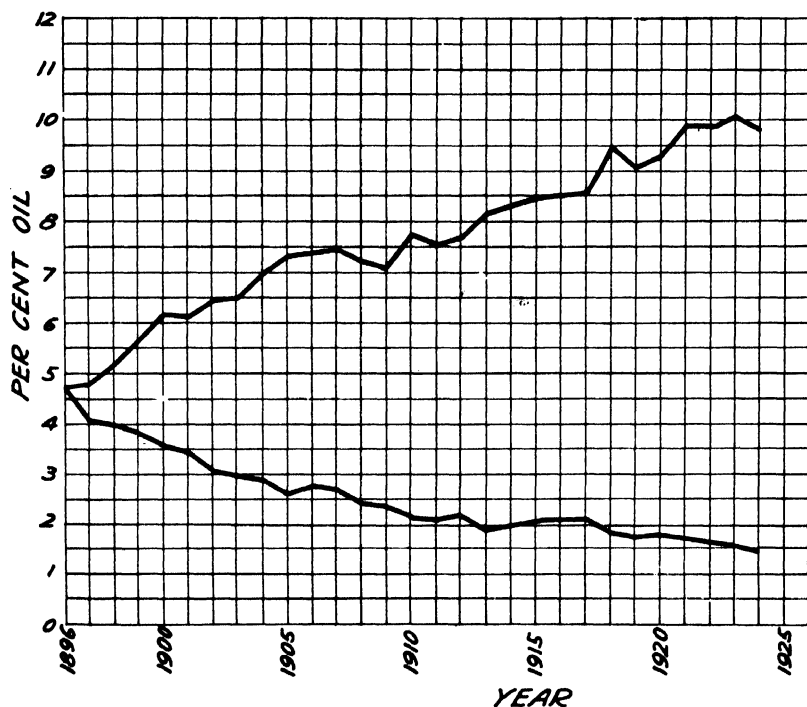


FIGURE 2.—Progress of high-oil and low-oil corn breeding

that were highest and lowest in protein or in oil had been selected for seed, regardless of the yielding ability of the detasseled rows. On the other hand, if selection had been carried on without taking yield into account, inbreeding might have resulted in such a loss of vigor that it would have been impossible to continue the strains. When it is remembered that the original purpose of the experiment was to produce good-yielding strains having the desired composition, any great reduction in yield would have defeated the purpose.

PEDIGREES

Graphic presentations of the pedigrees of the strains of corn used in this work follow.

PEDIGREE OF ILLINOIS HIGH-PROTEIN CORN

GENERATION NUMBER

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
																											01
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* These numbers, preceded by the generation numbers, denote the seed ear numbers.

PEDIGREE OF ILLINOIS LOW-OIL CORN

[illegible]

¹ These numbers, preceded by the generation numbers, denote the seed ear numbers.

A study of the pedigrees of the four strains shows that there has been a rapid elimination of lines until the four strains are now represented by only a single ear each of the original seed stock. The high-protein strain traces back to ear 121 which on analysis showed 12.28 per cent protein and 3.99 per cent oil. This is slightly below the average composition of 12.54 per cent for the stock seed ears but considerably above the average of the original 163 ears, 10.92 per cent. The low-protein strain goes back to ear 106, which on analysis yielded 8.25 per cent protein and 4.81 per cent oil. The average for the stock seed ears of the low-protein strain was 8.96 per cent. The high-oil strain goes back to ear 111, which on analysis showed 5.65 per cent oil and 10.82 per cent protein. The average for the stock seed ears for the high oil was 5.33 per cent as compared to 4.70 per cent, the average for the original 163 ears. The low-oil strain traces back to ear 110, which when analyzed was found to contain 4.10 per cent oil and 11.13 per cent protein. The average percentage of oil for the stock seed ears was 4.04. Thus, in the twenty-eighth year of selection all of the 96 ears of the four strains trace back to 4 ears of the original Burr's White.

Even though the ear has been used as the unit of selection and the history of the strains traced through the female side only, the reduction of the ancestry to a single ear for each strain indicates that the strains at the present date are likely to be more nearly homozygous than was the original material from which they came. Further evidence that the strains are more homozygous than open-pollinated varieties is furnished by selfing the strains. It has been shown at this station that upon selfing, a condition of uniformity is reached more quickly than with open-pollinated varieties. Although the individual inbred lines coming from any one of the strains differ among themselves in composition, they are always significantly different from any of the inbreds coming from the other strains.

That there is still some heterozygosity left in the individual within the strains is indicated by the fact that there is a reduction in vigor upon selfing.

East (2), in fitting curves to the data for the first 10 generations of selection, found that at first the curve was concave, showing great progress, later convex, showing that progress became slower, and at last horizontal, indicating that no more progress would result from selection. An inspection of Figures 1 and 2 presented here will show that, contrary to East's results, there has been considerable progress since the tenth generation for all of the strains with the possible exception of the Illinois low protein. The Illinois high-oil strain has shown greater progress since the tenth generation than it did before.

It does not seem possible to predict a limit to the progress that selection will make in the Illinois high-oil and the Illinois high-protein strains by the application of curves to the data at hand. Apparently the low-protein strain has made but little change in the last 20 years. If the average protein content for the years beginning with 1902 be compared with the protein content of ear 106, to which the strain now traces, no significant difference appears.

The low-oil strain is approaching a physiological limit. The greater percentage of the oil in a grain of corn is contained in the germ (9). Hence, in selecting for low-oil content the size of the germ has been decreased both absolutely and relatively in comparison with the size

of the endosperm. In the ear containing the lowest percentage of oil on record, namely, 0.69 per cent, 80 per cent of the grains were germless. The necessity of using ears having grains that will germinate naturally tends, therefore, to check the progress of selection, and eventually may stop it altogether.

EFFECT OF SELECTION UPON VARIABILITY

It seems to be accepted by most biologists that selection for a given character in a cross-fertilized crop like corn leads to a lower variability. That this reduction in variability may have its limits is brought out by Davenport (1) when he states that selection in a cross-fertilized crop such as corn simply shifts the type but does not appreciably change the variability unless a physiological limit is reached. As evidence for this belief, he cites Karl Pearson as stating that 10 to 13 per cent reduction in variability obtained by the selection of two parents is almost the limit that can be reached, even if the complete ancestry

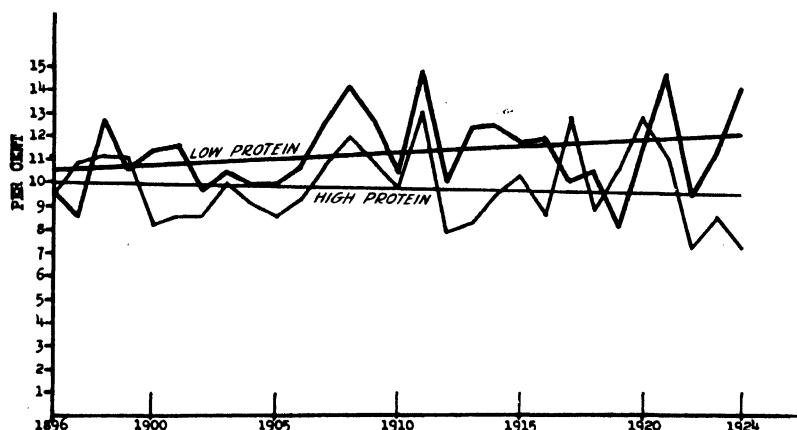


FIGURE 3.—Yearly variations in protein content of corn and their straight-line trends as measured by the coefficient of variation

had been selected. Davenport also uses the first eight years' data on the Illinois "chemical" strains of corn, employing the coefficient of variation as a measure of variability, and states that there is no significant change in variability. It is of interest to see how the matter stands after the results of 20 more years of continued selection have been secured.

VARIABILITY MEASURED BY THE COEFFICIENT OF VARIATION

PROTEIN

The effect of selection upon the variability in protein content as measured by the coefficient of variation from the beginning of the experiment through the 28 years of selection can be seen in Figure 3. Although the yearly variations as measured by this coefficient are rather large, there are decided trends that run in directions opposite to the line of selection. As selection leads to a low mean, the variability increases, and vice versa. As measured by the best fitting lines, the variability has increased 14.05 per cent in the low-protein strain over that at the beginning and decreased 4.83 per cent in the high-protein.

OIL

Similar results have been obtained for the oil content; only here the divergence is more marked. The variability, according to the coefficient of variability, has increased 69.84 per cent for the low-oil strain and decreased 23.21 per cent for the high-oil strain. (Fig. 4.)

It appears from these results that selection can change variability, as measured by the coefficient of variation, more than 13 per cent and that the variability may either increase or decrease, depending upon the magnitude of the mean. Although the coefficient of variation has been the most commonly used means of comparing variability when different types of variation are involved in the comparison, it is

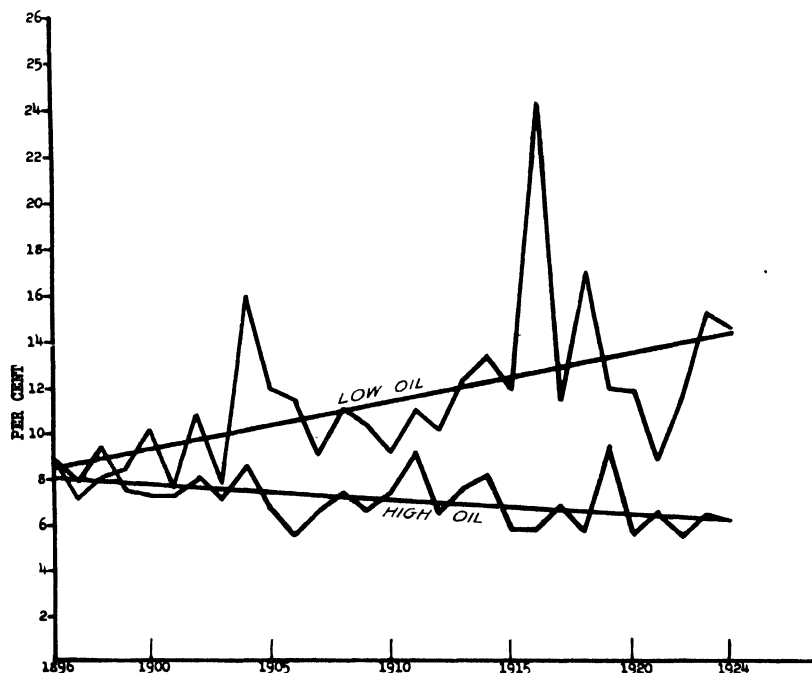


FIGURE 4.—Yearly variations in the oil content of corn and their straight-line trends as measured by the coefficient of variation

recognized to have decided limitations. Pearl (12) states that, "... the coefficient of variation has never been an entirely satisfactory constant to biologists, at least," and also that (11, p. 275) "one should always remember that this constant simply measures the degree of scatter of the distribution in relation to the mean value of the thing varying." Such a relation may have a real and significant meaning but sometimes it does not have, for reasons inherent in the nature of the facts themselves.

VARIABILITY MEASURED BY THE STANDARD DEVIATION

The standard deviation has usually been accepted as the standard method of measuring in absolute terms the degree of variability. It has the advantage that it is a constant of the mathematical formula for the curve of variation representing the distribution of a population.

PROTEIN

The variability for protein content in the Illinois high-protein and Illinois low-protein strains, as measured by the standard deviation (fig. 5), shows trends opposite to those obtained when the coefficient of variation is used to measure the variation. Although showing considerable yearly fluctuation, the variability tends to move in the same direction as the mean; e. g., the high-protein strain is more variable than the low-protein strain for protein content. During the

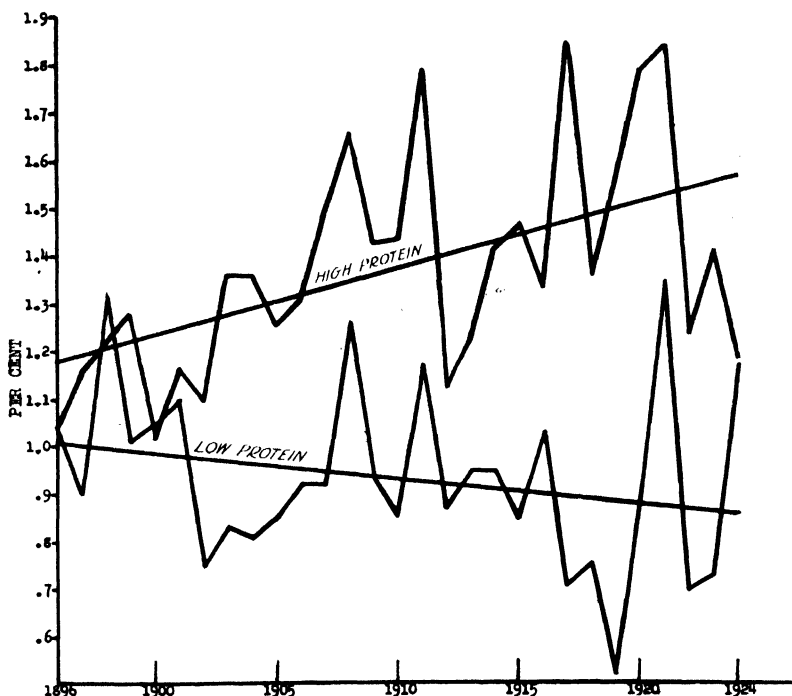


FIGURE 5.—Yearly variations in protein content of corn and their straight-line trends as measured by the standard deviation, 1896-1924

course of the experiments an increase in variability, according to this index, of 32.97 per cent for the high-protein strain and a decrease of 14.71 per cent for the low-protein strain have been obtained.

OIL

The variability measured by the standard deviation for the oil content shows similar results. (Fig. 6.) Here also the variability is greater in the strain having the higher content. An increase in variability of 50.95 per cent in the high-oil strain and a decrease of 36.44 per cent in the low-oil have been obtained. Again, the greater divergence in variability occurs between the oil strains.

When the standard deviation is used as a measure of variability, greater changes are exhibited than when the coefficient of variation is employed except for the low-oil strain. However, the standard

deviation has its limitations also. It can not be used to compare variability except where like things are measured in like units.

VARIABILITY MEASURED BY THE WEINBERG FORMULA

Weinberg (14) has recently proposed a method for measuring variation which is free from most of the limitations of the coefficient of variation and standard deviation. The coefficient $W =$

$$\frac{\sigma \sqrt{M_n - M_o}}{\sqrt{(M_a - M_o)(M_n - M_a)}}$$

when M_n is the highest value in the distribution, M_o is the lowest value in the distribution, M_a is the mean value of all variants. The denominator of the formula measures roughly the skewness of the variability curve, while the second term in the numerator measures

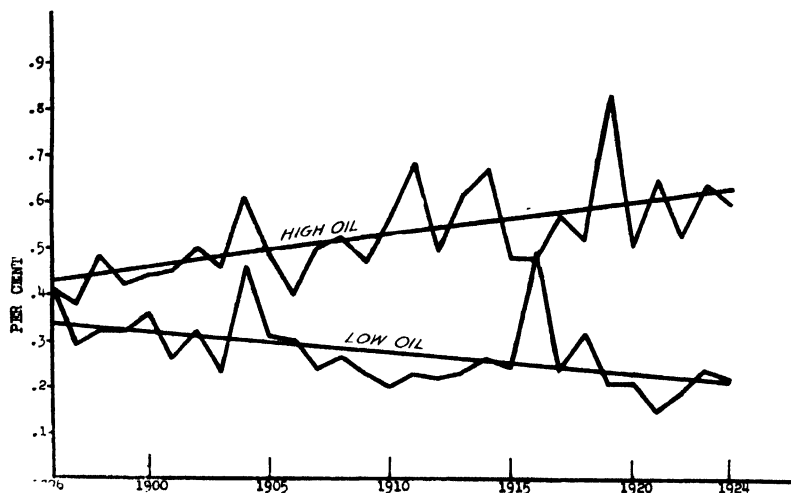


FIGURE 6.--Yearly variations in oil content of corn and their straight-line trends as measured by the standard deviation, 1896-1924

the range. The greater the skewness or the wider the range, or both, the greater the variability. The coefficient thus obtained is not affected by the magnitude of the mean as is the coefficient of variation, nor is it limited by unlike material or unlike units of measure as is the standard deviation. Like the coefficient of variation, however, it expresses variation as an abstract figure.

In Table 1 Weinberg's method is compared with the coefficient of variation for measuring variation by means of two examples. In example 1 the mean is 5, the standard deviation, 2.582, and the coefficient of variation is 51.64 per cent. By the Weinberg method the variation is 1.83. In example 2 each class is 10 units higher. The mean is 15, the standard deviation is the same as in example 1, but the coefficient of variation is 17.21 per cent. Variation, as expressed by Weinberg's formula, is the same as in example 1. It is believed that for comparative purposes the Weinberg formula gives a better conception of variability than does the coefficient of variation.

TABLE 1.—Comparison of coefficient of variation with Weinberg's formula for measuring variation

Example 1	Example 2
Class f	Class f
1 1	11 1
2 1	12 1
3 1	13 1
4 1	14 1
5 1	15 1
6 1	16 1
7 1	17 1
8 1	18 1
9 1	19 1
Mean = 5	Mean = 15
S. D. = 2.582	S. D. = 2.582
C. V. = 51.64%	C. V. = 17.21%
W. = 1.83	W. = 1.83

PROTEIN

The effect of selection upon variability for protein content, as measured by the Weinberg formula (fig. 7), is similar to the results

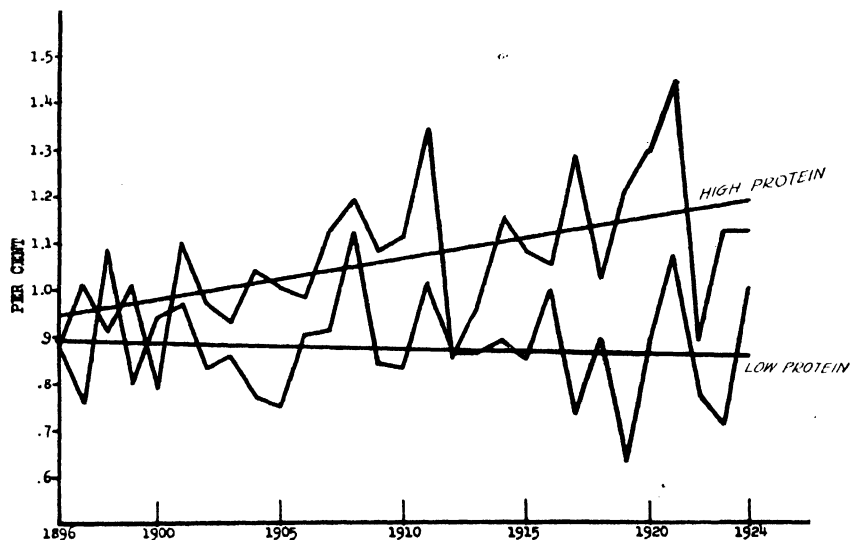


FIGURE 7.—Yearly variations in protein content of corn and their straight-line trends as measured by Weinberg's formula, 1896-1924

obtained when the standard deviation is used in that variability increases as selection leads to a high mean and decreases as selection leads to a low mean. The increase in variability for the high-protein strain was 25.49 per cent. However, the decrease in variability for the low-protein strain was only 3.94 per cent.

OIL

Similar results were obtained for variability in oil content of the oil strains. (Fig. 8.) The increase in the high-oil strain was 23.97 per cent and the decrease in the low-oil strain was 30.18 per cent. Again, the divergence in variability is greater between the oil strains than between the protein strains.

VARIABILITY MEASURED BY THE MODAL CLASS

Since the type of the population is represented by the modal class, it is of interest to know what proportion of the population resides within the modal class as selection continues.

A graphical representation of the modal classes in respect to composition for the four different strains taken at periodic intervals is seen in Figure 9. The blocks represent the relative proportion of the population residing in the modal class for the respective years. Although there is considerable fluctuation, it can be seen that the trend is downward for the high-oil and the high-protein strains, i. e., as selection leads toward a higher mean fewer and fewer individuals of the population reside within the modal class. In the low-oil strain there is a decided trend upward. The trend in the

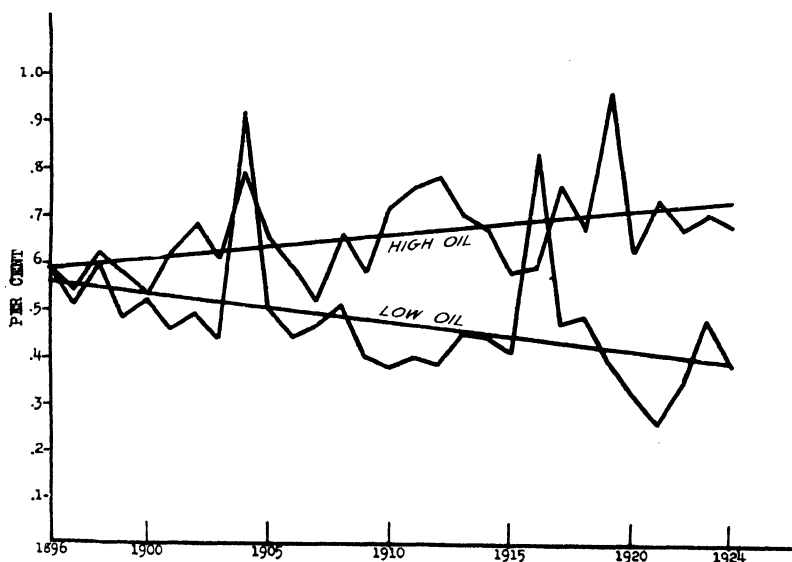


FIGURE 8.—Yearly variations in oil content of corn and their straight-line trends as measured by Weinberg's formula, 1896-1924

low-protein strain is slightly upward. Hence, selection for a low mean increases the percentage of individuals lying in the modal class.

The percentage of the population in the modal class may be taken as a rough measure of the uniformity, and, correspondingly, the percentage outside of the modal class as an expression of variability. In general, the greater the percentage of the population lying in the modal class the less the variability, and vice versa. This variability may be expressed in the form of a ratio $\frac{100-Y}{Y}$, where Y is the percentage of the population lying in the modal class. The smaller Y is the greater the ratio; hence, the greater the variability.

Variability may also be expressed by the inverted straight-line trend for the modal classes. For the purposes of this work such trends were determined by the use of the formulas $\frac{100-Y_o}{Y_o} = X_o$.

and $\frac{100 - Y_a}{Y_a} = X_a$ when Y_o is the origin and Y_a the destination of the best fitting straight line for the modal classes, and X_o and X_a are the origin and destination, respectively, for the lines to be determined. The quotient thus obtained may be called the extramodal coefficient because it takes into account the population outside of the modal class. The trends thus obtained (figs. 10, 11, 12, 13) indicate that variability becomes less as a low mean is approached and greater as a high mean is approached. The method is empirical and therefore has the limitations that most empirical formulas have.

COMPARISON OF THE DIFFERENT MEASURES OF VARIABILITY

A comparison of the four different measures of variability discussed above for each of the four strains is shown in Figures 10, 11, 12, and 13. The standard deviation and the coefficient obtained by the Weinberg method are multiplied by 10 so that they may be plotted on a common scale with the other measures. It is to be noted that

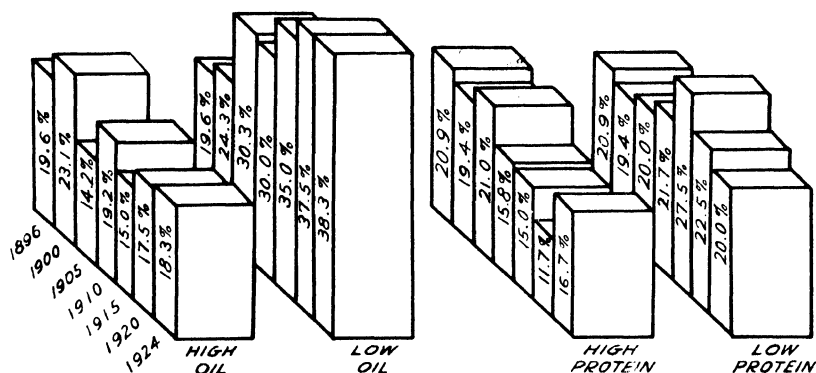


FIGURE 9.—Graphic presentation of the modal classes with respect to the percentage composition of Illinois high and low oil, and high and low protein corn, at periodic intervals between 1896 and 1924

in all cases variability progresses in the same direction as the mean when measured by the standard deviation, Weinberg formula, and the extramodal coefficient. If variability is expressed as a percentage of the mean it decreases as the mean increases, and vice versa. Of the four strains, the low protein shows on an average the least change in variability. (Table 2.)

RELATIVE VARIABILITY IN PROTEIN AND OIL

The protein strains show greater variability than do the oil strains (Tables 3-6), thus suggesting that the former may be affected to a greater extent by the environment. Hopkins states (8 p. 239) "the fat content of corn is even more susceptible to the influence of seed selection than is the protein content, doubtless due to the fact that the primary materials from which fat is manufactured, namely, carbon dioxide and water, are usually furnished to the plant in unlimited supply, while the formation of protein is essentially dependent upon the supply of available nitrogen in the soil." Variability is usually augmented when the living material under study is grown at the minimum or maximum rather than at the optimum condition.

TABLE 2.—Comparison of change in variability for chemical composition in four Illinois strains of corn as determined by the best fitting lines for four different measures of variability, 1896 and 1924

Strain	Method used to measure variability	Year		Percentage of increase (+) or decrease (-)
		1896	1924	
High protein	Coefficient of variation	10.03	9.54	* -4.86
	Standard deviation	1.18	1.57	+33.05
	Weinberg's formula	.95	1.19	+25.26
	Extramodal coefficient	3.79	6.37	+68.07
Low protein	Coefficient of variation	10.53	12.02	+14.15
	Standard deviation	1.01	.86	-14.85
	Weinberg's formula	.89	.86	-3.37
	Extramodal coefficient	4.19	3.14	-25.06
High oil	Coefficient of variation	8.11	6.23	-23.18
	Standard deviation	.43	.64	+48.84
	Weinberg's formula	.59	.73	+23.73
	Extramodal coefficient	4.09	5.02	+22.74
Low oil	Coefficient of variation	8.50	14.44	+69.88
	Standard deviation	.34	.21	-38.24
	Weinberg's formula	.56	.39	-30.36
	Extramodal coefficient	3.68	1.50	-59.24

* These figures differ slightly from those in the text. The figures in the text were based on four figures after the decimal.

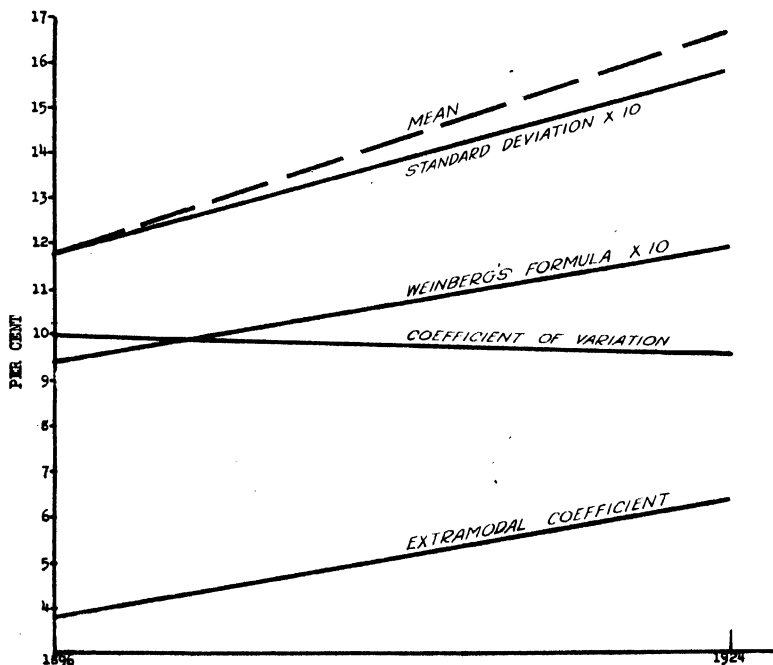


FIGURE 10.—Comparison of four different measures of variation for protein content of Illinois high-protein corn as expressed by the best fitting straight lines; the broken line shows the trend of the mean protein content

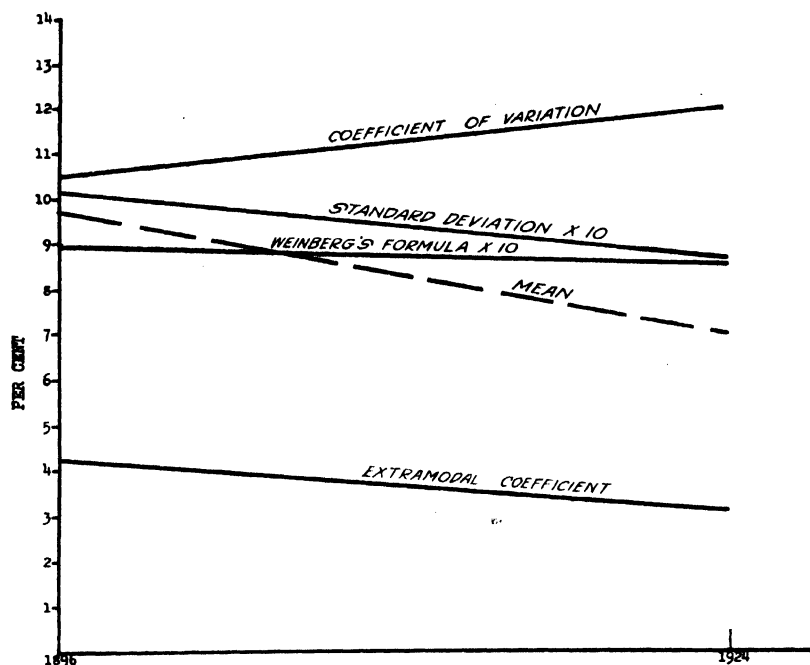


FIGURE 11.—Comparison of four different measures of variation for protein content of Illinois low-protein corn as expressed by the best fitting straight lines; the broken line shows the trend of the mean protein content

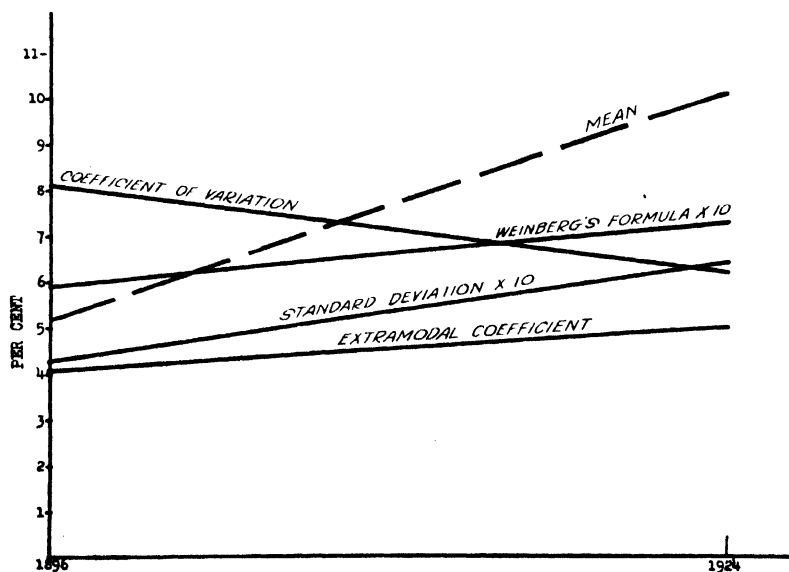


FIGURE 12.—Comparison of four different measures of variation for oil content of Illinois high-oil corn as expressed by the best fitting lines; the broken line shows the trend of the mean oil content

TABLE 3.—Average protein content and its percentage variability in Illinois high-protein strain corn, 1896-1924, as measured by different methods, together with extreme variates

Year	Ears analyzed	Average protein content	Standard deviation	Coefficient of variation	Variability by Weinberg's formula	Lowest variate	Highest variate	Population in modal class
	Number	Per cent			Per cent			Per cent
1896	103	^a 10.93	^a 1.04	^a 9.50	0.881	8.3	13.9	20.9
1897	112	10.99	1.16	10.90	1.008	8.3	13.6	
1898	252	10.98	1.22	11.15	.912	7.7	14.9	
1899	216	11.62	1.28	11.00	1.012	8.4	14.8	
1900	216	12.62	1.02	8.09	.795	9.3	15.7	19.4
1901	114	13.78	1.17	8.48	1.103	11.5	16.0	
1902	90	12.90	1.10	8.50	.965	9.5	15.0	
1903	100	13.51	1.36	10.04	1.025	8.5	17.3	
1904	100	15.03	1.36	9.05	1.040	10.6	17.8	
1905	119	14.73	1.26	8.55	.998	10.8	17.4	21.0
1906	120	14.26	1.31	9.19	.978	10.5	17.7	
1907	120	13.90	1.49	10.72	1.119	10.3	17.4	
1908	119	13.94	1.66	11.91	1.193	9.4	17.3	
1909	120	13.29	1.43	10.76	1.076	9.2	16.4	
1910	120	14.87	1.44	9.68	1.109	11.2	18.0	15.8
1911	120	13.79	1.79	12.98	1.344	10.3	17.4	
1912	120	14.49	1.13	7.80	.854	10.3	17.5	
1913	120	14.83	1.22	8.23	.965	11.6	18.0	
1914	120	15.04	1.42	9.44	1.148	11.5	17.8	
1915	120	14.54	1.47	10.19	1.076	10.7	18.2	15.0
1916	120	15.66	1.34	8.56	1.047	12.7	19.3	
1917	120	14.45	1.85	12.80	1.277	10.2	18.6	
1918	120	15.49	1.36	8.78	1.022	11.8	18.9	
1919	120	14.70	1.55	10.54	1.201	11.1	17.8	
1920	120	14.01	1.79	12.78	1.287	9.5	17.4	11.7
1921	120	16.66	1.84	11.04	1.441	9.4	18.8	
1922	120	17.34	1.24	7.15	.890	12.6	20.6	
1923	120	16.53	1.41	8.50	1.116	13.1	19.7	
1924	120	16.60	1.19	7.17	1.120	14.6	19.2	16.7

^a Data for the years 1896-1903 are taken from Davenport's Principles of Breeding (1, p. 446) means and standard deviations for the remaining years are calculated by the nongrouping method.

TABLE 4.—Average protein content and its percentage variability in Illinois low-protein strain corn, 1896-1924, as measured by different methods, together with extreme variates

Year	Ears analyzed	Average protein content	Standard deviation	Coefficient of variation	Variability by Weinberg's formula	Lowest variate	Highest variate	Population in modal class
	Number	Per cent			Per cent			Per cent
1896	163	^a 10.93	^a 1.04	^a 9.50	0.881	8.3	13.9	20.9
1897	60	10.63	.90	8.47	.759	8.2	14.0	
1898	126	10.49	1.32	12.61	1.089	7.5	13.4	
1899	144	9.59	1.01	10.50	.802	6.7	13.1	
1900	144	9.13	1.04	11.34	.937	7.1	12.3	19.4
1901	126	9.63	1.10	11.47	.975	7.6	13.1	
1902	90	7.86	.75	9.60	.829	6.4	9.7	
1903	100	8.00	.83	10.41	.862	6.4	10.2	
1904	100	8.17	.81	9.91	.773	6.1	10.5	
1905	120	8.58	.85	9.91	.753	6.6	12.1	20.0
1906	120	8.65	.92	10.64	.899	6.7	10.9	
1907	120	7.32	.92	12.57	1.910	5.8	10.5	
1908	120	8.96	1.26	14.06	1.117	6.3	11.4	
1909	120	7.48	.84	12.57	.827	5.5	10.8	
1910	120	8.26	.86	10.41	.822	6.5	11.0	21.7
1911	120	7.90	1.17	14.81	1.005	5.9	12.1	
1912	120	8.23	.82	9.96	.860	6.8	10.8	
1913	120	7.71	.95	12.32	.861	5.7	10.8	
1914	120	7.67	.95	12.39	.890	5.9	10.8	
1915	120	7.27	.85	11.69	.854	5.7	9.9	27.5
1916	120	8.68	1.03	11.86	.994	6.6	10.9	
1917	120	7.09	.71	10.01	.233	5.6	9.6	
1918	120	7.13	.75	10.52	.894	5.9	8.8	
1919	120	6.46	.52	8.05	.629	5.4	8.3	
1920	120	7.54	.89	11.80	.890	6.0	10.5	22.5
1921	120	9.14	1.35	14.77	1.074	6.6	13.4	
1922	120	7.42	.70	9.43	.774	6.1	9.6	
1923	120	6.48	.73	11.27	.708	5.0	9.4	
1924	120	8.38	1.17	13.96	.998	6.1	11.8	20.0

^a Data for the years 1896, 1898, 1899, 1900, 1901, 1902, and 1903 are taken from Davenport's Principles of Breeding (1, p. 448); means and standard deviations for the remaining years are calculated by the nongrouping method.

TABLE 5.—Average oil content and its percentage variability in Illinois high-oil strain corn, 1896-1924, as measured by different methods, together with extreme variates

Year	Ears analyzed	Average oil content	Standard deviation	Coefficient of variation	Variability by Weinberg's formula	Lowest variate	Highest variate	Population in modal class
	Number	Per cent			Per cent			Per cent
1896	163	4.68	0.41	8.83	0.585	3.9	6.0	19.6
1897	80	4.79	.38	7.87	.543	3.6	5.7	
1898	216	5.10	.48	9.33	.615	4.1	6.7	
1899	108	5.65	.42	7.47	.582	4.3	6.5	
1900	108	6.10	.44	7.26	.527	4.6	7.4	23.1
1901	126	6.24	.45	7.26	.621	4.9	7.1	
1902	90	6.25	.50	8.06	.680	5.0	7.2	
1903	100	6.51	.46	7.07	.608	5.5	7.6	
1904	101	7.11	.61	8.61	.792	6.0	8.4	
1905	120	7.30	.49	6.70	.651	6.3	8.6	11.2
1906	120	7.38	.40	5.46	.594	6.6	8.5	
1907	120	7.43	.51	6.82	.522	6.2	8.7	
1908	120	7.21	.53	7.36	.658	5.9	8.5	
1909	120	7.05	.47	6.62	.580	5.8	8.4	
1910	120	7.72	.57	7.36	.718	6.5	9.0	19.2
1911	120	7.52	.69	9.20	.763	5.9	9.2	
1912	120	7.71	.50	6.49	.778	6.5	8.7	
1913	120	8.16	.62	7.63	.700	6.4	9.6	
1914	120	8.30	.68	8.23	.668	5.8	10.1	
1915	120	8.47	.49	5.77	.576	6.9	9.8	15.0
1916	120	8.51	.49	5.80	.592	7.2	10.0	
1917	120	8.52	.58	6.78	.782	7.2	9.7	
1918	120	9.36	.53	5.67	.674	8.0	10.5	
1919	120	9.06	.84	9.26	.961	7.3	10.4	
1920	120	9.28	.52	5.57	.619	7.8	10.6	17.5
1921	120	9.04	.66	6.64	.729	8.4	11.7	
1922	120	9.86	.64	5.48	.674	8.7	11.3	
1923	120	10.08	.65	6.45	.685	8.3	11.8	
1924	120	9.86	.61	6.19	.676	8.4	11.7	18.3

* Data for the years 1896-1903 are taken from Davenport's principles of breeding (1, p. 446); means and standard deviations for the remaining years are calculated by the nongrouping method.

TABLE 6.—Average oil content and its percentage variability in Illinois low-oil strain corn, 1896-1924, as measured by different methods, together with extreme variates

Year	Ears analyzed	Average oil content	Standard deviation	Coefficient of variation	Variability by Weinberg's formula	Lowest variate	Highest variate	Population in modal class
	Number	Per cent			Per cent			Per cent
1896	163	4.68	0.41	8.83	0.585	3.9	6.0	19.6
1897	50	4.10	.29	7.10	.510	3.4	4.7	
1898	108	3.59	.32	8.13	.589	3.2	4.8	
1899	144	3.85	.32	8.42	.484	2.8	4.6	
1900	144	3.57	.36	10.13	.522	2.6	4.5	24.4
1901	126	3.45	.26	7.59	.456	2.8	4.1	
1902	90	3.00	.32	10.83	.492	2.4	3.8	
1903	90	2.99	.23	7.83	.441	2.5	3.6	
1904	100	2.89	.46	15.91	.920	2.4	3.4	
1905	119	2.58	.31	11.86	.502	1.8	3.1	30.3
1906	120	2.67	.30	11.35	.443	1.6	3.5	
1907	120	2.60	.24	9.04	.466	2.2	3.3	
1908	120	2.39	.27	11.09	.507	1.8	2.9	
1909	120	2.24	.23	10.40	.402	1.4	2.8	
1910	120	2.21	.20	9.05	.384	1.6	2.7	30.0
1911	120	2.06	.23	11.02	.398	1.4	2.7	
1912	120	2.19	.22	10.05	.386	1.3	2.7	
1913	120	1.91	.24	12.30	.451	1.3	2.4	
1914	120	1.98	.26	13.33	.446	1.3	2.7	
1915	120	2.07	.25	11.93	.410	1.4	3.1	35.0
1916	120	2.10	.50	24.30	.828	1.3	4.7	
1917	120	2.10	.24	11.38	.474	1.7	3.0	
1918	120	1.88	.32	16.86	.480	1.3	2.5	
1919	120	1.77	.21	11.92	.304	1.3	2.5	
1920	120	1.80	.21	11.83	.323	1.0	2.4	37.5
1921	120	1.71	.15	8.77	.264	1.0	2.3	
1922	120	1.68	.19	11.55	.347	.9	2.2	
1923	120	1.58	.24	15.19	.480	1.1	2.1	
1924	120	1.51	.22	14.57	.387	.9	2.2	38.3

* Data for the years 1896, 1898, 1899, 1900, 1901, 1902, and 1903 are taken from Davenport's Principles of Breeding (1, p. 446); means and standard deviations for the remaining years are calculated by the nongrouping method.

SYMMETRY OF DISTRIBUTION

A number of other statistical expressions have been proposed intended to describe the nature of a population with respect to its distribution. Certain of these expressions are of interest in connection with the present study. An important item of information in regard to a distribution is whether the variates are symmetrically distributed with reference to the mean or whether there is a bunching of variates on one side of the mean and a long tailing out of the variates on the other side; i. e., to know the amount of skewness.

Another item that should be known is whether the variates are densely grouped at the mean, giving a high peak to the frequency

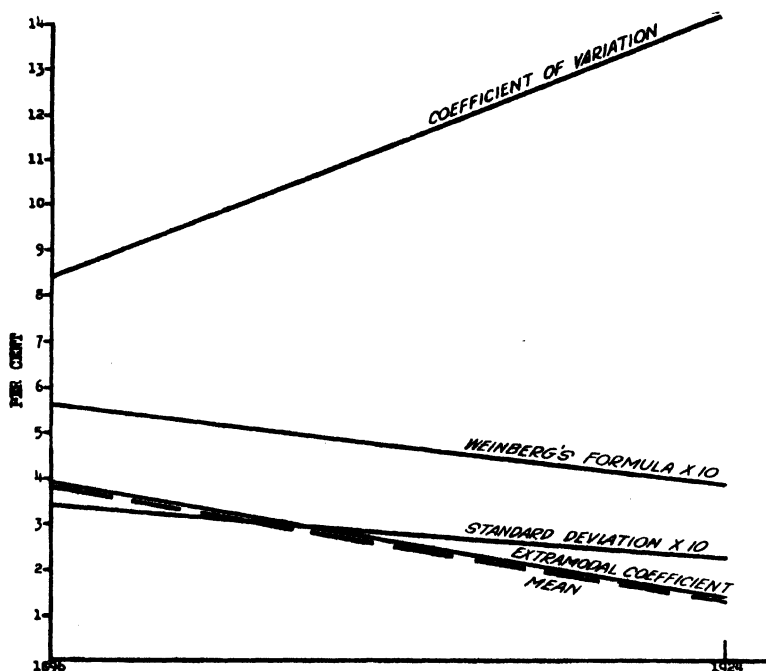


FIGURE 13.—Comparison of four different measures of variation for oil content of Illinois low-oil corn as expressed by the best fitting straight lines; the broken line shows the trend of the mean oil content

polygon, or whether the distribution is rather flat in the middle and contracted at the ends, or whether the distribution of the variates is intermediate between these two conditions; i. e., to know the amount of kurtosis. A normal distribution is said to be mesokurtic, a peaked curve leptokurtic, and a flat curve platykurtic.

The mean, median, mode, standard deviation, coefficient of variation, variation by Weinberg's formula, skewness, and kurtosis are given in Table 7 for each of the four strains taken at periodic intervals. The median, mode,⁴ skewness,⁵ and kurtosis⁶ were calculated

⁴ Mode = Mean - 3.03 (mean - median).

⁵ Skewness = $P_{.90} - P_{.10} - \frac{1}{2}D$. S. D. of Sk = $0.59914 \sqrt{\frac{D}{N}}$. $D = P_{.90} - P_{.10}$.

⁶ Kurtosis = $\frac{Q}{D}$. Q = quartile deviation. S. D. of Ku. = $\frac{0.27779}{\sqrt{N}}$.

on the basis of percentiles.⁷ Kelley (10, p. 58-62, 75-77) states that "this method of determining curve types, although in general not as accurate as the longer method of Pearson, can be used where the populations are large and the standard errors are small." The variates of the high-oil and the low-oil strains appear to be fairly symmetrically distributed with reference to the mean. In no case is skewness significantly different from zero. In five of the seven years studied the median is greater than the mean in the high-oil strain. In the low-oil strain the median is lower than the mean in four of the seven years. The differences in all cases are very small.

TABLE 7.—Measurements of central or average tendencies, and of dispersions for distributions of the variates of four Illinois strains of corn taken at periodic intervals

HIGH-PROTEIN STRAIN									
Year	Number of ears analyzed	Mean	Median	Mode	Standard deviation ^a	Coefficient of variation ^a	Variability by Weinberg's formula ^a	Skewness ^b	Kurtosis ^c
1896	163	10.96	11.04	11.18	1.04	9.50	0.88	+0.1082±0.0874	-0.0008±0.0147
1900	216	12.69	12.64	12.55	1.02	8.09	.80	- .1312± .0704	+ .0101± .0127
1905	119	14.77	14.94	15.29	1.26	8.55	1.00	+ .3997± .2312	- .0320± .0172
1910	120	14.93	15.08	15.37	1.44	9.68	1.11	+ .2159± .1313	+ .0229± .0171
1915	120	14.55	14.67	14.92	1.47	10.19	1.08	+ .2447± .1426	+ .0106± .0171
1920	120	14.04	13.92	13.67	1.79	12.78	1.29	- .2885± .1775	+ .0206± .0171
1924	120	16.68	16.65	16.58	1.19	7.17	1.12	- .1027± .1237	- .0065± .0171
LOW-PROTEIN STRAIN									
1896	163	10.96	11.04	11.18	1.04	9.50	0.88	+0.1082±0.0874	+0.0008±0.0147
1900	144	9.19	9.07	8.83	1.04	11.34	.94	- .2044± .0622	+ .0043± .0156
1905	120	8.64	8.48	8.15	.85	9.91	.75	- .1772± .0946	+ .0398± .0171
1910	120	8.32	8.24	8.08	.86	10.41	.83	- .1443± .0870	- .0085± .0171
1915	120	7.30	7.16	6.85	.85	11.69	.85	- .2182± .0775	+ .0018± .0171
1920	120	7.60	7.50	7.29	.89	11.80	.89	- .1543± .0835	+ .0061± .0171
1924	120	8.44	8.34	8.14	1.17	13.96	1.00	- .1865± .1049	- .0050± .0171
HIGH-OIL STRAIN									
1896	163	4.74	4.83	5.00	0.41	8.83	0.59	+0.0323±0.0391	-0.0368±0.0147
1900	108	6.15	6.20	6.31	.44	7.26	.53	+ .0654± .0653	- .0051± .0180
1905	120	7.35	7.34	7.32	.49	6.70	.65	- .0053± .0519	+ .0330± .0171
1910	120	7.78	7.72	7.60	.57	7.36	.72	- .1090± .0507	- .0274± .0171
1915	120	8.52	8.58	8.69	.49	5.77	.58	+ .1098± .0574	+ .0014± .0171
1920	120	9.34	9.35	9.38	.52	5.57	.62	+ .0850± .0491	- .0192± .0171
1924	120	9.92	10.00	10.17	.61	6.19	.68	+ .0117± .0726	- .0301± .0171
LOW-OIL STRAIN									
1896	163	4.74	4.83	5.00	0.41	8.83	0.59	+0.0323±0.0391	-0.0368±0.0147
1900	144	3.63	3.61	3.56	.36	10.13	.52	- .0565± .0304	+ .0007± .0152
1905	119	2.64	2.62	2.58	.31	11.86	.50	- .0197± .0262	+ .0250± .0172
1910	120	2.26	2.27	2.29	.20	9.05	.38	+ .0260± .0241	- .0171± .0171
1915	120	2.13	2.12	2.12	.25	11.93	.41	- .0177± .0209	+ .0155± .0171
1920	120	1.84	1.86	1.89	.21	11.83	.32	+ .0257± .0227	- .0268± .0171
1924	120	1.56	1.54	1.52	.22	14.57	.39	- .0130± .0240	- .0322± .0171

^a Taken from Tables 3, 4, 5, and 6.

^b A positive sign (+) indicates that the distribution tails out on the low side.; a negative sign (-) indicates the reverse.

^c Data reported equals Ku. less 0.26315. If sign is positive (+) curve is platykurtic; if sign is negative (-) curve is leptokurtic.

⁷ Determine the class in which the $pN+\frac{1}{2}$ measure lies.

Let f_p = the frequency in this class.

Let i_p = the interval or range covered by this class.

Let F_p = the sum of the frequencies in all classes below this class.

Let v_p = the value of the lower boundary of this class.

Let N = the total population.

Let P_p = percentile, the value of which is to be calculated.

Let p = proportion of classes having values smaller than P_p .

Then $P_p = v_p + \frac{pN - F_p}{f_p} i_p$.

The distributions for the low-protein strain are negatively skewed, i. e., tail out on the high side for each of the years studied except 1896. Although the skewness is much greater than in the case of the oil strains, in no instance is skewness significantly different from zero. Likewise, the distributions for the high-protein strain show a greater skewness than do the distributions for the oil strains, but in no case is the skewness significantly different from zero.

The four strains show but little deviation from a mesokurtic curve. The small deviations from the normal probability curve are as frequent in the direction of a leptokurtic curve as of a platykurtic curve.

A study of the extreme variates of the different strains for the period of selection (fig. 14) shows that in the high-oil strain the variates deviate as far on the low side as they do on the high side. In the low-oil strain the deviation is slightly greater on the high side.

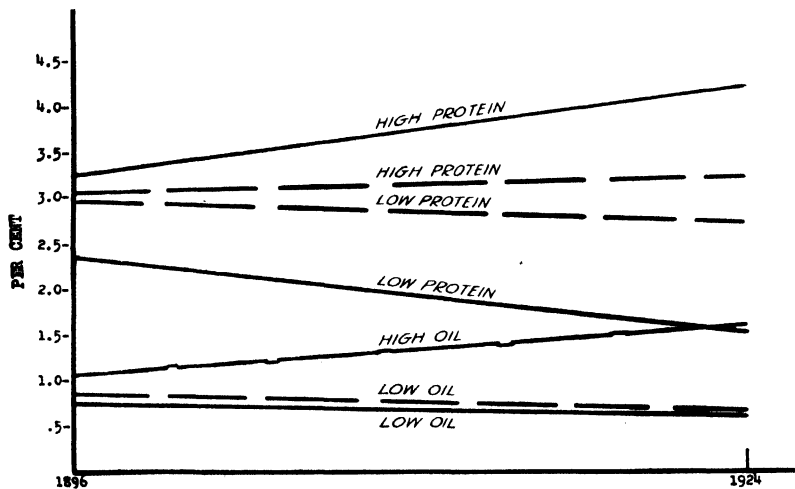


FIGURE 14.—Comparison of the range between the lowest variate and the mean (solid line), and the highest variate and the mean (broken line), for high-protein, low-protein, high-oil, and low-oil Illinois corn as expressed by the best fitting straight lines

However, the extreme variates in the high-oil strain are about twice as far from the mean as those in the low-oil strain. The range in the low-oil strain is becoming less while that in the high-oil strain is becoming greater.

The extreme variates in the low-protein strain are farther from the mean in a positive direction than in a negative direction, while in the high-protein strain the reverse is true. This means that the distribution tails out toward the mean of the original nonselected material in both cases.

GENERAL DISCUSSION

If, as has been stated by many, the population with the greatest variability offers the greatest chance for improvement by selection then it should be possible to make still greater progress in the Illinois high-protein and Illinois high-oil strains in future years than has been made in the past because the range of each was greater in 1924

than it was in 1896. (Fig. 15.) Such a conclusion would be rather questionable. As has been stated, the strains now appear to be more nearly homozygous than was the original material, and our knowledge of genetics and the effects of selection would lead us to believe that they should be. Being more nearly homozygous, they should be less variable genetically. The apparent increase in variability must then be due either to the environment or to the methods of measuring variability.

Emerson (4, p. 30-31) in speaking of the variability in number of rows on an ear of corn says: ". . . It is more reasonable to suppose

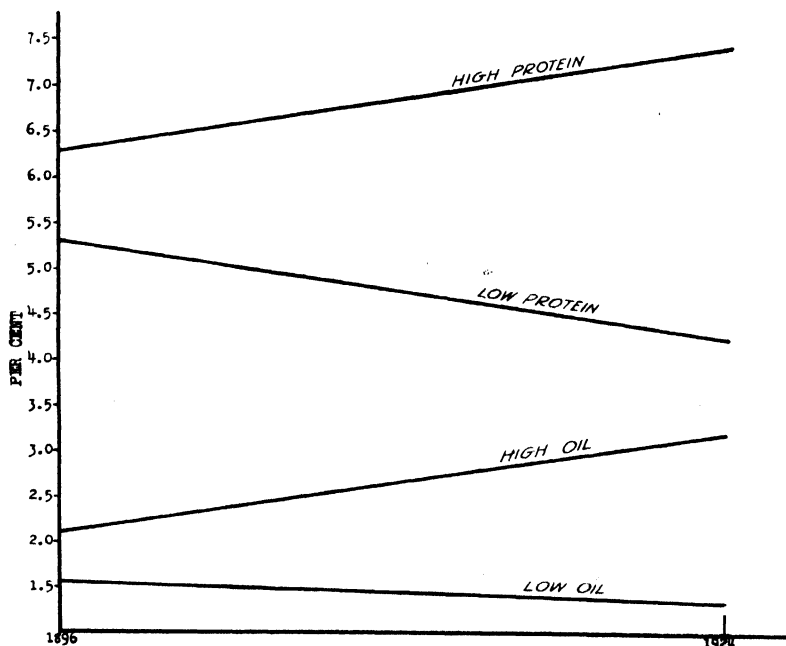


FIGURE 15.—Comparison of the ranges between the highest variate and the lowest variate for high-protein, low-protein, high-oil, and low-oil Illinois corn as expressed by the best fitting straight lines

that an ear which can vary in any one of eight spikes will show a greater degree of fluctuation than one which can vary only in any one of four spikes. For this reason it is likely that strains with a high number of rows will never show the low variability seen in strains with a low number of rows." One may reason in like manner about variability of the oil and of the protein content for the different strains. The high strains are more variable than the low strains because there is more material present for the environment to interact with. Supposing the germ of the high-oil strain to contain 600 cells and that of the low oil to contain 300 cells, we might expect twice the effect on the high-oil strain from environmental action as on the low oil under identical conditions.

Zeleny (15, p. 15) states: "It is a common principle of embryology that a changed condition does not act by accretion, i. e., by addition

or subtraction of individual parts without affecting the rest. On the contrary, the action is upon all of the preexisting parts of the organ." This he calls the theory of proportionate action. Zeleny, in the study of the effect of selection for eye facet number in the white bar-eye race of *Drosophila melanogaster*, found that a race with 300 facets was affected ten times as much as a race with only 30 facets by a 1° change in temperature. In order to measure the variability of races with different facet numbers he scaled his classes for grouping so that each class range was a fixed percentage of the mean of its class. He used as a mid or zero point the mean of the original population from which his selected material came. He (15, p. 15) goes on to explain that "the standard deviation, as determined by this method, is expressed in factorial units and serves directly as a coefficient of variation strictly comparable in all cases, regardless of the mean values of the different stocks that may be compared."

Should such a method of measuring variability be applied to the distributions of the oil and protein strains in the present study, it can be readily seen that the degree of deviation from the means would depend upon the value taken as a percentage of the means of the classes. The larger this figure the smaller the deviation of the high strain as compared to the low strain. Also, the farther the high strain is removed from the low strain the greater will be its comparative reduction in variability. The factorial method of measuring variability has not been used in an analysis of these data because the method affords no manner of determining the figure to be used in arriving at the class ranges with such data. Such a figure might be 10, 20, or 23 per cent. Zeleny apparently uses such a figure as will give a normal distribution for the populations studied. As has been shown, the distributions for protein and for oil content thus far studied do not show a significant deviation from the normal curve.

Although it appears logical to attribute the increase in variation in the high strains to the fact that there is more material for the environment to interact with, it is impossible to prove it, because the variability due to the environment can not be separated from the variability due to the segregation and recombination of factors. The complexity of the inheritance of protein and oil content and the manner of conducting the selection work make it entirely impossible to analyze the four strains genetically. Others who have studied the protein content of corn have also found it impossible to determine the genetic factors involved. East (3) lists a large number of factors other than genetic that may and probably do affect protein content, e. g., number of seeds on the ear, size of pericarp, lack of phosphorus, and departure from optimum temperature and moisture at critical periods. In addition to these there may be considered the factors affecting size of germ, size of endosperm, absorption of different amounts of food elements through the roots and their translocation through the stalk and subsequent deposition within the pericarp. The total protein content is made up chemically of at least four protein groups (13) each of which may be represented by a single or several genes in the germ plasm. Some of these factors may be dominant and others recessive, or there may be any gradation of dominance. Hayes (5) states: "Protein content is therefore inherited in

much the same way as other characters which are dependent for their full expression on many different inherited factors of the plant and likewise upon environmental conditions." What has been said of the protein content may likewise be said of the oil content.

SUMMARY

Twenty-eight years of continuous selection for protein and oil content in corn has produced four types which are distinctly different in their composition. When compared with the original nonselected material the high-protein and the high-oil strains show a proportional increase of 50.01 and 109.79 per cent, respectively. The low-protein and low-oil strains show a proportional decrease of 23.26 and 67.87 per cent, respectively.

The high-protein and high-oil strains show no indications of having reached a limit to further increases.

The low-protein strain has changed but little during the last 20 years.

The low-oil strain is approaching a physiological limit to further decreases. Ears with extremely low oil content have a high percentage of germless seeds.

The four different strains now trace back in their pedigrees to a single ear each.

Variability has been shown to change considerably following selection. The degree of change in variability depends somewhat upon the method used in measuring it.

Variability of oil or protein content appears to depend upon the magnitude of the mean of the selected character.

Variability as measured by the coefficient of variation increases when selection leads to a low mean, and vice versa.

Variability, as measured by the standard deviation, Weinberg's formula, and extramodel coefficient, increases when selection leads to a high mean, and vice versa.

The percentage of the population lying in the model classes decreases when selection leads to a high mean, and vice versa.

The symmetry of the distribution curve as determined by the percentile method for the four strains taken at periodic intervals is not significantly different from that of the normal variability curve.

It is suggested that the apparent increase in variability of the high strains may be due to the fact that there is more material present for the environment to interact with.

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A BOTANICAL AND CHEMICAL STUDY OF BIKUKULLA EXIMIA, WITH A KEY TO NORTH AMERICAN SPECIES OF BIKUKULLA¹

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INTRODUCTION

In 1921 a study was made of the alkaloids of *Bikukulla cucullaria* and *B. canadensis* in relation to their poisonous properties to grazing animals² and from the former there was isolated a new and extremely toxic alkaloid to which was given the name cucullarine. *B. canadensis* was also found to be poisonous but to a less degree. In view of these results it was thought worth while to make a chemical study of *B. eximia*, since no such study had previously been reported.

All the members of the *Bikukulla* group on which chemical work has been reported are known to contain alkaloids, which is not surprising since they are closely allied to the poppy family. Few of the alkaloids, however, have been examined with sufficient care to prevent confusion as to their identity, and, with the exception of protopine, dicentrine, cucullarine, and an unnamed alkaloid isolated from *B. canadensis*, practically nothing is known about their toxicity.

BIKUKULLA EXIMIA

BOTANICAL DESCRIPTION

Bikukulla eximia (Ker) Millsp. Fringed bleedingheart. Also known as wild bleedingheart, staggerweed, and turkey corn. (Fig. 1.) Perennial, smooth herb, with dark-green basal leaves ternately compound, finely cut into oblong or ovate segments; scaly rootstocks; flower pedicels rising from the root 1 to 2 feet high, taller than the leaves, in compound lengthened clusters; flowers flattened, usually withering and persistent; sepals 2, scalelike; petals 4, the outer produced into rounded sacs at the base, 2 inner petals crested; stamens 6, in two groups, their filaments often united; pods several seeded, splitting; seeds black, warty, crested. Habitat, mountain rocks and river gorges.

This species was found at Junius, Seneca County, N. Y., by H. P. Startwell more than a century ago, but it has never been found so far north since. Otherwise the plant ranges in the southern Appalachian Mountains from Wills Mountain, Allegany County, Md., south to Georgia.

EXPERIMENTAL WORK

The material on which the work was done consisted of leaves and twigs of *Bikukulla eximia* collected near Deerfield in Augusta County, Va., by the senior writer in June, 1923. This material, which was in

¹ Received for publication Mar. 19, 1929; issued October, 1929.

² BLACK, O. F., EGGLESTON, W. W., KELLY, J. W., and TURNER, H. C. POISONOUS PROPERTIES OF BIKUKULLA CUCULLARIA (DUTCHMAN'S-BREECHES) AND B. CANADENSIS (SQUIRREL-CORN). Jour. Agr. Research 23: 69-78, illus. 1923.

good condition when received, was ground to a moderately fine powder. A few grams of the powdered product was extracted with Prollius's solution, and the evaporated extract was taken up with



FIGURE 1.—*Bikukulla eximia* in flower. $\times \frac{1}{4}$

dilute hydrochloric acid, filtered, and tested with Mayer's reagent. This gave a heavy flocculent precipitate, indicating the presence of alkaloids in quantity.

As the primary object of the work was to find out whether this plant is toxic to animals, the next step was to make a preparation that would contain the total alkaloids from a known weight of the plant and to test the toxicity of the preparation by administering definite doses of it to white mice. To this end 30 gm. of the finely ground plant was moistened with alcohol and acetic acid, packed in a percolator, and thoroughly extracted with 95 per cent alcohol. The resulting extract was evaporated by distillation under reduced pressure, and the residue was taken up with dilute acetic acid and then made alkaline with ammonia. The alkaloid was shaken out with chloroform, which entirely removed it. The chloroform extract was concentrated to a few cubic centimeters, and then extracted with dilute acetic acid. The last extract was made alkaline with ammonia and again shaken out with chloroform, the solvent was evaporated in a tared dish, and the residue was weighed. The residue consisted of an amorphous light-yellow substance weighing 0.7 gm., equivalent to 2.3 per cent of the dry plant. The crude alkaloid was next treated with very dilute hydrochloric acid, which, it was observed, did not effect complete solution. The insoluble portion was dried and weighed. One-half cubic centimeter of the solution, representing 8 mgm. of alkaloid, was then injected subcutaneously into a mouse weighing about 15 gm. No marked symptoms of any kind were observed in the animal, with the possible exception of a slight drowsiness. As 8 mgm. is a very large dose for an animal of 15 gm. weight, it was concluded that the alkaloids contained in *Bikukulla eximia* were of slight physiological activity.

The portion of the crude total alkaloid that did not dissolve in the hydrochloric acid weighed 0.19 gm. and represented approximately 15 per cent of the whole. This was redissolved in a little alcohol and ammonia, and on the addition of water the free alkaloid separated in fine white needle crystals. The crystals were washed on a filter with water, dried, and redissolved in alcohol, in which they were readily soluble, and purified by conversion into the chloride and recovery of the free base. As thus prepared the compound was found to have a melting point of 165° C. (uncorrected) and consisted of colorless needles, very soluble in alcohol and chloroform but insoluble in water. With gold and platinum chlorides it gave only amorphous precipitates, but with picric acid it formed a finely crystalline salt which could be recrystallized from hot alcohol and which had a melting point of 175° C. (uncorrected). The alkaloid, therefore, shows every evidence of its chemical individuality and is differentiated from other alkaloids hitherto found in this group of plants by its insoluble hydrochloric acid salt as well as by other physical properties. There is every reason, then, to believe it to be a new alkaloid, and the name *eximine* is proposed for it.

The physiological action of this new alkaloid was tested by dissolving a weighed quantity in very dilute acetic acid and injecting the solution subcutaneously into a white mouse. A dose of 3 mgm. produced only a slight restlessness, which soon wore off, and no further symptoms could be detected. This alkaloid, therefore, is not of a dangerously poisonous character. As the other alkaloidal contents of the plant were likewise found to be innocuous, it seems safe to conclude that *Bikukulla eximia* should not be classed as a poisonous plant.

Table 1 summarizes the chief chemical and physical characteristics of the alkaloids of the *Bikukulla* group that have been reported, including eximine, the new compound that has been obtained from *B. eximia*.

TABLE 1.—Comparison of the characteristics of previously reported alkaloids of *Bikukulla* species with those of *B. eximia*

Alkaloid	Species in which the alkaloid occurs	Melting point (° C.)	Solubility	Form
Protopine	<i>B. cucullaria</i>	206-208.	Ether chloroform	Needles and prisms.
	<i>B. pusilla</i>			
	<i>B. formosa</i>			
	<i>B. spectabilis</i>			
Dicentrine	<i>B. pusilla</i>	168-169.	Hot alcohol chloroform.	Prisms.
Cucullarine	<i>B. cucullaria</i>	168.	Ether chloroform	Do.
Eximine	<i>B. eximia</i>	165.	Alcohol chloroform	Needles.
Unnamed	<i>B. canadensis</i>	210.	Alcohol	Silky, yellow needles.
Do.	<i>B. cucullaria</i>	235.	Alcohol (insoluble)	Needles.
Do.	do.	215.	Alcohol (soluble)	Granular.
Do.	<i>B. formosa</i>	168.5.	Alcohol (insoluble)	Yellow needles.
Do.	do.	142.5.	Alcohol (soluble)	White needles.

Alkaloid	Color tests				Physiological action
	Concentrated H ₂ SO ₄	Erdman's	Froede's	Concentrated HNO ₃	
Protopine	Yellow	Yellow to violet	Violet	Colorless	Narcotic.
Dicentrine	Violet	Blue	Deep blue	do.	Toxic.
Cucullarine	Yellow	Colorless	Green to blue	Orange	Very toxic.
Eximine	Crimson	Fale yellow	do.	Brown	Nontoxic.
Unnamed	Light yellow		Green	Orange	Do.
Do.	Red to brown	Red to violet	Red to violet	Red to yellow	
Do.	Violet	Blue	Deep blue	Colorless	
Do.	Colorless	Green	Blue green	Brown	

FIELD NOTES ON THE GENUS BIKUKULLA

Bikukulla is largely a North American group of plants, with three species in the East (*B. cucullaria*, *B. canadensis*, and *B. eximia*) and several species on the Pacific coast.

An extended field survey was made in the mountain counties of southwestern Virginia in April, 1921, and in May and June, 1923. *Bikukulla eximia* was not observed in 1921, but in 1923 it was collected at Deerfield and also on the Peaks of Otter. *B. cucullaria* and *B. canadensis* were found in abundance in many places in Giles, Bland, Tazewell, Russell, and Washington Counties. They are more abundant in these localities than anywhere else in their range, but they were even more abundant before the forests were generally removed.

A better field knowledge of the western species is being acquired with a view to obtaining material for chemical study. *Bikukulla formosa*, the first species discovered on the Pacific slope, was observed by the senior writer in a number of places in the Sierra Nevada in California, but only a few plants at a time. This species is much more abundant in the moist woods along the streams flowing into Puget Sound and is common in the vicinity of Glacier, Wash. *B. chrysantha*, one of the yellow-flowered species of California, ranges

from central to southern California and is abundant in Bull Run Canyon, Sequoia Forest, Kern County. Three other species (*B. ochroleuca*, *B. uniflora*, and *B. pauciflora*) are of less importance and as yet have not been observed in any quantity. The western form of *B. cucullaria*, considered by many botanists as a distinct species, and perhaps rightly so, should be studied chemically in comparison with the eastern species.

KEY TO NORTH AMERICAN SPECIES OF BIKUKULLA

Stems leafy, tall; flowers yellow; corolla deciduous; seeds crestless.

Flowers sulphur yellow; outer petals spreading or recurving to the middle; widely scattered. California and southern Oregon . . . 1. *B. chrysantha*.

Flowers straw yellow or cream colored; outer petals erect or only tips spreading; Santa Ynez Mountains to the Santa Monica Mountains.

2. *B. ochroleuca*.

Stems naked; leaves basal; flowers white or pink, nodding; seeds crested.

Rootstocks tuber bearing; racemes simple.

Rootstocks long, with scattered cornlike yellow tubers; flowers cordate; spurs rounded 3. *B. canadensis*.

Rootstocks much shortened; tubers at base of stem.

Tubers gathered in a scaly, granulated bulb, white to dark red; flowers several; sagittate; spurs long, spreading. 4. *B. cucullaria*.

Tubers in a fascicle, elongate, tapering toward the base; flowers 1 or 2, outer petals long, tips recurving beyond the body; small; alpine.

5. *B. uniflora*.

Rootstocks not tuber bearing.

Racemes simple; flowers 1 to 3; petals distinct, outer petals narrow, recurved; small; alpine. 6. *B. pauciflora*.

Racemes compound.

Appalachian species; flowers oblong; crest of inner petals projecting.

7. *B. eximia*.

Pacific coast species; corolla ovate cordate; petals united. 8. *B. formosa*.

RELATION OF PICKING TIME TO ACETALDEHYDE CONTENT AND CORE BREAKDOWN OF BARTLETT PEARS¹

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INTRODUCTION

In the course of studies designed to throw light upon the factors associated with the production of scald and breakdown in Bartlett pears, it was reported (1) ² that acetaldehyde was formed as a product of vital activity of pear protoplasm and was present in the tissues in increasing amounts during the period of ripening. The close correlation that was found to exist between relatively high quantities of acetaldehyde in the tissues and the occurrence of scald and breakdown suggested that acetaldehyde may be instrumental in the initiation of these disorders. More recent investigations have substantiated the earlier findings and have yielded further information regarding the behavior of Bartlett pears in relation to acetaldehyde content and breakdown.

It has been observed (2) ³ that maturity at the time of harvest is an important factor in the keeping quality of the pear. When pears are picked late in the season breaking down at the core is more prevalent than when they are picked earlier, and in general the later the time of removal from the tree the more pronounced the disease becomes. Breakdown will also occur on pears allowed to ripen immediately upon their removal from the tree without being subjected to storage conditions. Scald, on the other hand, is more prevalent on pears picked immaturity and does not appear normally until the fruit has been in storage for some time (2, 4).⁴

Magness and Ballard (5), studying the respiration of Bartlett pears, found that the rate of ripening seemed to parallel the carbon dioxide output, that early picked fruit respired at a lower initial rate, and that this rate was accelerated less rapidly than that of late-picked fruit from the same tree. They also stated that Bartlett pears which were ripened at temperatures around 60° F. showed a marked increase in the concentration of carbon dioxide in the tissues and a considerable decrease in oxygen.

In the light of these observations it was considered important to make a further study of the acetaldehyde content of Bartlett pears, its relation to breakdown as influenced by the time of harvesting, and the carbon dioxide and oxygen concentrations within the tissues.

¹ Received for publication Mar. 7, 1929; issued October, 1929.

² Reference is made by number (italic) to "Literature cited," p. 493.

³ DIEHL, H. C. PICKING PEARS IN RELATION TO QUALITY. 10 p. [Mimeographed.] (Circular for the use of the inspectors of the cooperative Federal and Washington State shipping-point fruit and vegetable inspection service.)

⁴ See footnote 3.

MATERIALS AND METHODS

In the experiments described in this paper, only Bartlett pears were used. These were obtained from two trees which were especially selected because of their similarity in location, growth, and size of crop. The fruit was picked on four different dates to represent the entire harvest period of the Wenatchee (Wash.) district, where the fruit for the experiment was obtained: August 9, 1927, the early commercial picking date; August 17, the early to midseason maturity stage; August 26, the midseason to late-season stage; and September 12, the late stage.

The pears of each picking were divided into three equal portions and placed under the following conditions: (1) Immediately ripened at 22°–24° C.; (2) stored at 0° C. for one month before being ripened at 22°–24° C.; (3) stored at 0° C. for two months and subsequently ripened at 22°–24° C. The fruit at 0° C. was held in a modern commercial cold-storage plant maintaining a fairly constant temperature with the maximum fluctuations not exceeding 2° C. In the ripening room thermographic records showed a fairly uniform range of 22°–24° C.

Samples for chemical analysis and maturity tests were taken at the time of picking, and samples for chemical analysis at intervals throughout the ripening periods. Five pears were selected at random for acetaldehyde determination. These were cut into equal pieces and samples of tissue for analysis weighed from these portions. Seeds and carpel walls were carefully excluded from the samples, and pears showing any visible evidence of scald were discarded. Separate analyses of the intercellular gas of three to five pears were made, and the individual determinations were averaged.

CHEMICAL ANALYSES

The method employed for the determination of acetaldehyde is to be found in a recent article (1). Samples for carbon dioxide and oxygen analyses were taken from the pears at right angles to the long axis with a large cork borer. From these samples the intercellular gases were extracted by means of a modification of the apparatus described by Magness (3). The gases were collected over mercury and transferred to a Bonnier-Mangin gas-analysis apparatus.

MATURITY TESTS¹

Possibly the most accurate and satisfactory method of determining the maturity of pears is by the use of the pressure tester, a device which measures the resistance of the flesh of the fruit to mechanical pressure in terms of pounds. The instrument used in this investigation is described in Department Circular No. 350 (6). Standard color charts were also used in the maturity tests.

PRESENTATION OF DATA

Data secured from the chemical analyses and the maturity tests are presented in Tables 1, 2, and 3. In these tables are given the amounts of acetaldehyde in the tissues, the percentages of carbon

¹ For these data the writer is indebted to H. C. Diehl and L. A. Fletcher, Bureau of Plant Industry, U. S. Department of Agriculture.

dioxide and oxygen in the intercellular gases, the resistance of the tissues to pressure in pounds, and the average color of the fruit at the time of harvesting and at intervals during the ripening period. In Table 1 the results are from the pears picked on the four different dates and placed immediately in the ripening room at 22°-24° C. In Tables 2 and 3 are presented the analyses of the pears after periods of one and two months, respectively, in cold storage at 0° C. and then placed in the ripening room at 22°-24° C. Samples for analysis were taken upon the removal of the pears from cold storage and at intervals during the ripening period.

TABLE 1.—*Analyses of Bartlett pears ripened at 22°-24° C. immediately after picking*

Picking date (1927)	Days in ripening room	Acetalde- hyde in 100 gm. of fresh tissue	Composition of intercellular gas		Average resistance to pressure	Average color ^a
			Carbon dioxide	Oxygen		
			Per cent	Per cent		
		<i>Milli- grams</i>			<i>Pounds</i>	
Aug. 9.....	0	0	3.0	16.2	17.6	1.0
	2	.30				
	10	1.24	9.2	17.1		
	20	11.66	15.0	14.2		
	^b 24	14.28	5.7	17.4		
	^c 30	13.22	(^d)	(^d)		
Aug. 17.....	0	0	3.5	15.6	16.2	1.5
	2	.35	4.4	17.1		
	12	5.39	14.2	16.0		
	16	13.82	14.9	13.9		
	^b 22	13.79	9.9	16.4		
	^c 28	11.00	(^d)	(^d)		
Aug. 26.....	0	0	3.8	15.0	15.4	2.0
	2	.44	6.3	18.0		
	7	.72	11.5	14.7		
	13	11.29	16.0	12.0		
	^b 19	20.10	15.2	14.2		
	^c 24	14.40	(^d)	(^d)		
Sept. 12.....	0	0	10.6	13.4	11.0	2.5
	2	.66	15.4	12.3		
	^b 7	14.50	14.5	15.2		
	10	14.80	24.8	11.1		
	^e 15	15.40	(^d)	(^d)		
	^f 12	12.27				

^a Color was determined according to the method used by Magness, Diehl, and Allen (7, p. 3).

^b Slight browning about vascular and core tissues.

^c Browning about core tissues and extending into the cortex; fruit wilted.

^d Fruit too soft to sample.

^e Tissues badly broken down; no wilting.

^f Badly broken down.

TABLE 2.—Analyses of Bartlett pears placed in cold storage at 0° C. immediately after picking, and held there for one month before being ripened at 22°–24° C.

Picking date (1927)	Days in ripening room	Acetalde- hyde in 100 gm. of fresh tissue	Composition of intercellular gas		Average resistance to pressure	Average color
			Carbon dioxide	Oxygen		
		Milli- grams	Per cent	Per cent	Pounds	
Aug. 9.....	0	0.22	2.9	20.6	16.3	2
	4	1.21	14.5	12.4		
	7	6.22	15.9	12.6		
	13	8.36	16.9	14.5		
	19	10.45	32.4	6.8		
Aug. 17.....	^a 25	10.00	13.3	13.2	15.7	2
	0	.29	3.5	20.0		
	4	1.32	22.1	8.6		
	6	5.39	26.8	6.0		
	12	10.23	21.8	10.8		
Aug. 26.....	^a 18	10.56	18.1	14.5	14.4	2.5
	^b 24	10.01	4.5	19.1		
	0	.11	16.9	12.4		
	4	1.54	28.6	8.7		
	9	6.27	30.4	7.5		
Sept. 12.....	^c 14	19.58	21.1	12.5	11.4	4
	^d 18	13.53	5.0	21.1		
	0	4.18	26.9	7.5		
	3	9.35	32.6	8.6		
	^e 7	16.50	20.7	10.8		
	^e 10	20.90	31.6	5.8		
	^d 13	13.53				

^a Core tissues slightly browned; slight wilting.^b Browning of the core and cortex tissues; badly wilted.^c Core and cortex tissues broken down.^d Badly broken down.^e Badly broken down; no wilting.

TABLE 3.—Analyses of Bartlett pears placed in cold storage at 0° C. immediately after picking, and held there for two months before being ripened at 22°–24° C.

Picking date (1927)	Days in ripening room	Acetalde- hyde in 100 gm. of fresh tissue	Composition of intercellular gas		Average resistance to pressure	Average color
			Carbon dioxide	Oxygen		
		Milli- grams	Per cent	Per cent	Pounds	
Aug. 9.....	0	0.20	3.7	20.0	16.7	2
	4	1.32	14.7	14.5		
	9	11.22	28.9	9.7		
	^a 18	13.53	22.1	10.2		
	^b 22	7.92	6.1	19.9		
Aug. 17.....	0	.55	8.7	17.0	14.6	2
	1	1.76	23.6	9.6		
	3	8.36	21.5	4.4		
	^a 13	11.44	19.2	10.7		
	17	10.00	16.4	14.0		
Aug. 26.....	^b 22	9.68	18.1	11.7	14.5	3
	0	.55	5.0	19.2		
	5	6.27	28.7	8.8		
	^a 9	14.74	31.2	9.5		
	^d 14	17.71	21.0	13.2		
Sept. 12.....	17	11.22	31.5	8.0	11.0	4
	^e 0	11.66	6.6	19.9		
	2	13.97	14.1	13.5		
	4	16.61	15.6	15.6		
	6	19.25	27.5	10.3		
	^d 9	21.45	13.0	15.0		
	11	23.94	18.3	12.0		
	^a 13	19.91	17.0	10.8		
	15	13.85	17.1	7.4		

^a Slight browning of the core tissues; slightly wilted.^b Badly wilted and broken down.^c Core tissues broken down; no wilting.^d Core and cortex tissues badly broken down.^e All tissues completely broken down.

BREAKDOWN IN RELATION TO MATURITY

From the data presented in Tables 1 to 3 it is apparent that the stage of maturity at the time of harvesting has a decided influence upon the subsequent keeping quality of Bartlett pears. This is reflected in the length of time elapsing from the date they were removed from the tree until they showed the first evidence of breakdown. In Table 4 there are shown the number of days required to produce breakdown in the various pickings when the fruit was ripened at 22°-24° C. As evidenced there, the earlier the pears were picked the longer was the time required for breakdown to occur. This was true not only for the pears ripened immediately after picking, but also for those ripened after storage.

TABLE 4.—*Number of days required for Bartlett pears to show first evidence of breakdown when ripened at 22°-24° C.*

Picking date (1927)	Ripened immediately	Ripened after being held in cold storage for—	
		1 month	2 months
	Days	Days	Days
Aug. 9	24	25	18
Aug. 17	22	18	13
Aug. 26	19	14	9
Sept. 12	7	7	(a)

^a Pears showed slight core breakdown when removed from cold storage.

These results agree with the findings of Hartman (2), Diehl,⁶ and others, who contend that core breakdown may not be a commercial factor if proper attention is paid to maturity at the time of harvest. Diehl⁶ states that, in the Northwest, Bartlett pears picked at a pressure ranging from 20 to 16 pounds have given a product of good shipping character and acceptable quality when ripened, but if picked below 16 pounds there is danger of core breakdown. Evidence obtained in the present investigation confirms this observation.

One of the physical factors which may play an important rôle in the relation between breakdown and maturity is wilting. High air humidity unquestionably retards wilting, but in these experiments, where all the lots were held under comparable conditions, the later picked fruit showed little or no wilting, while that picked early was found to show excessive wilting when ripened at 22°-24° C.

RELATION OF ACETALDEHYDE TO BREAKDOWN

In a previous paper (1) it was stated that acetaldehyde is a product of protoplasmic activity during the ripening period of Bartlett pears, and that comparatively large quantities of this substance are present whenever the pears exhibit scald or breakdown. In the present study the original conclusions have been confirmed so far as breakdown is concerned. In this investigation attention was directed to breakdown only, for, as has already been stated, pears ripened immediately after removal from the tree do not normally develop scald, and it

⁶ See footnote 3.

was considered important in this work to include aldehyde development in pears that had not been subjected to cold-storage conditions. Therefore, for the sake of strict interpretation, any pears subsequently developing scald were discarded.

As shown in Table 1, pears analyzed immediately upon removal from the tree showed no trace of acetaldehyde in any of the pickings. However, after a period of two days in the ripening room, the tissues contained distinct quantities. From this time on there was an increase of aldehyde, reaching a maximum about the time breakdown was evident, as determined by browning of the cells.

In Tables 2 and 3 analyses are given at the ends of 1-month and 2-month storage periods, respectively. It can be seen that during storage at 0° C. acetaldehyde accumulated in all lots. In the fourth picking the aldehyde content was distinctly greater than in the earlier

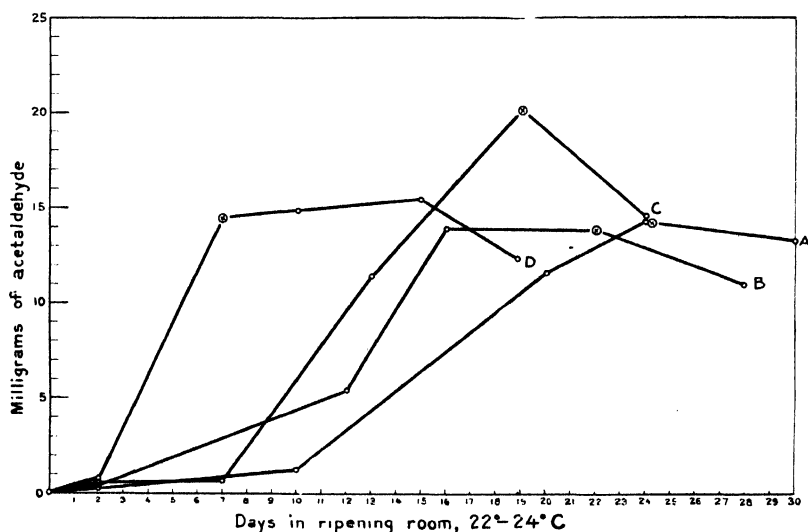


FIGURE 1.—Progress of acetaldehyde accumulation in Bartlett pears ripened at 22°-24° C. immediately after picking. Fruit was analyzed at time of picking and at intervals during the ripening period. Curve A represents acetaldehyde content of fruit picked August 9; B, August 17; C, August 26; and D, September 12

picked fruit. At the end of two months' storage the late picked pears began to show evidences of breakdown, and in these pears the concentration of aldehyde was high, even while they were in cold storage. In these cold-storage lots, as in the immediately ripened lots, aldehyde accumulation took place within the tissues when the fruit was ripened at 22°-24° C. and reached comparatively high concentrations when breakdown occurred.

A comparison of the aldehyde data of Tables 1, 2, and 3 is shown graphically in Figures 1, 2, and 3, respectively. In each of the figures, curves A, B, C, and D represent the progress of acetaldehyde accumulation through the ripening period of pears picked August 9, August 17, August 26, and September 12, respectively, from the time they were removed from the tree or from cold storage until complete breaking down of the tissues occurred. The point at which breakdown was evident is indicated on each curve by an encircled x.

Graphic illustrations are here presented of the relation existing between the concentration of acetaldehyde within the tissues of ripening pears and the occurrence of breakdown as influenced by the maturity of the fruit at the time of harvest. A striking feature of these curves is the acetaldehyde increment as influenced by the time of picking. In general, the later the fruit was picked the more rapid was the accumulation of acetaldehyde. This is especially true of the two later pickings as illustrated by curves C and D. In the earlier pickings, curves A and B, the aldehyde values tend to merge and overlap after the cold-storage periods.

The longer period required for breakdown to appear in the early-picked pears, and the comparatively smaller amounts of acetaldehyde present in the tissues at the time of breakdown in these early picked lots, apparently carry some special significance. This condition

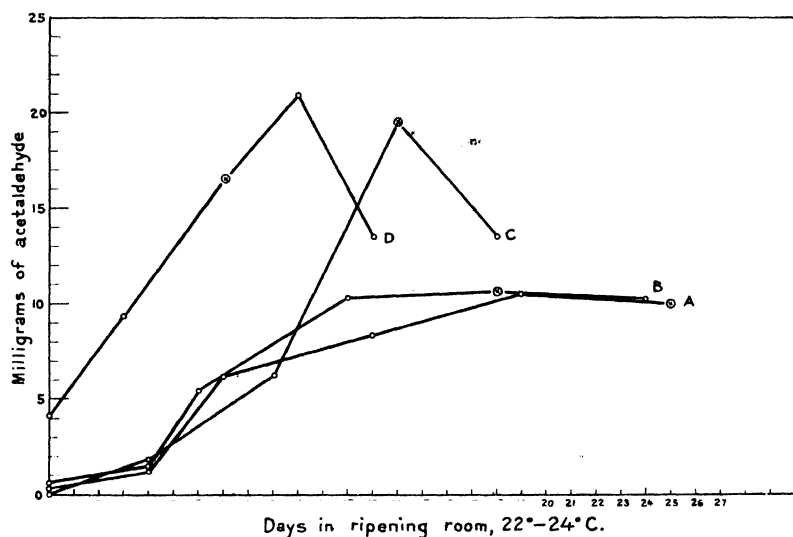


FIGURE 2.—Progress of acetaldehyde accumulation in Bartlett pears placed in cold storage at 0° C. immediately after picking, and held there for one month before being ripened at 22°-24° C. Fruit was analyzed immediately upon removal from cold storage and at intervals during the ripening period. Curve A represents acetaldehyde content of fruit picked August 9; B, August 17; C, August 26; and D, September 12.

exists mainly in the fruit ripened after being held in cold storage, but it is also found to a lesser degree in that ripened immediately after harvesting. It would seem, therefore, that, if acetaldehyde is a causative agent in the production of breakdown, the concentrations of this substance would be present in the tissues in equal amounts when breakdown occurred, regardless of any of the other attending factors. This, however, is not the case, and it is evident that some factor or group of factors must be responsible for the higher concentrations of aldehyde in the late picked pears. Two hypotheses are here suggested to account for this difference in behavior. First, as the pear matures on the tree, the mechanism responsible for the production of acetaldehyde is more highly developed than in immature fruit. There is evidence to show that acetaldehyde in apple tissues is the product of a zymase system (8), and such an enzyme system may occur in

pears. In fact, results obtained during the course of this and other investigations indicate that this is the case. If zymasic enzymes are produced or activated during ripening, the greater aldehyde concentration in the late picked lots could thus be accounted for.

The second hypothesis is based on the physical changes that occur in the tissue structures as maturity advances. In Table 1, 2, and 3 it is noted that the early picked pears showed considerable wilting while in the ripening rooms. Certain differences in skin structure are probably of special importance in connection with this wilting. The development of corky cells in the lenticels and the accumulation of waxlike substances on the surface of the skin are no doubt responsible for the greater water-holding power of the late picked fruit. The immature or early picked pears, because of the lack of this skin development, gives up moisture more readily and in addition probably

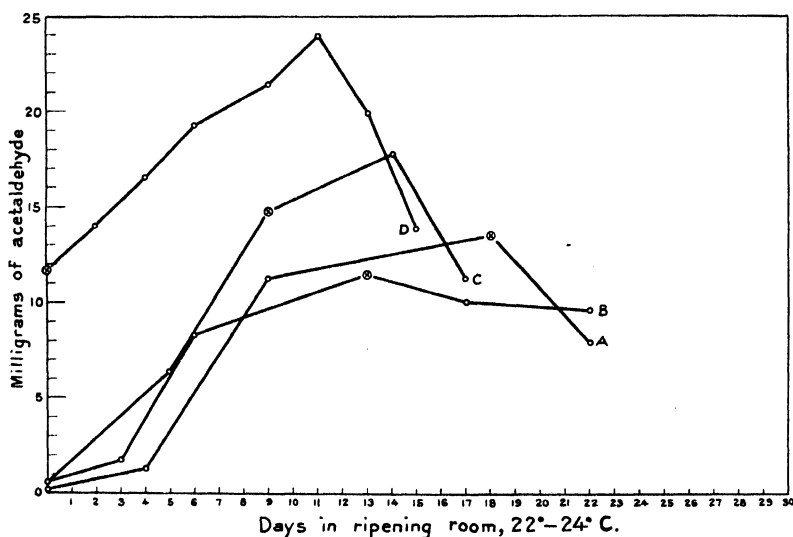


FIGURE 3.—Progress of acetaldehyde accumulation in Bartlett pears placed in cold storage at 0° C. immediately after picking, and held there for two months before being ripened at 22°-24° C. Fruit was analyzed immediately upon removal from cold storage and at intervals during the ripening period. Curve A represents acetaldehyde content of fruit picked August 9; B, August 17; C, August 26; and D, September 12

offer less resistance to gaseous exchange. It is possible that the tissues of mature fruit, which are more capable of retaining moisture, present a better anaerobic environment for zymasic activity and hence have a greater aldehyde production. It is also possible that the immature fruit may allow the gaseous aldehyde to escape more readily because of the more permeable nature of their outer tissues.

If there is an outward movement of acetaldehyde in immature pears the prevalence of scald in early picked fruit may be accounted for by the continued exposure of the skin cells to the escaping aldehyde, or these tissues may be more susceptible to injury than the cells of the cortex. The fact that some fruits that do not show tissue browning contain equal or higher concentrations of aldehyde than others that do show injury may be the result of the ability of certain cells to resist the toxic effects of the aldehyde. It is possible that

exposure to slight concentrations for a time may develop a temporary immunity in certain tissues.

These factors either separately or in combination may account for the variations that occur in the acetaldehyde content of the early picked and late picked pears. Possibly the greatest limitation in the interpretation of the production and fate of acetaldehyde is the lack of quantitative measurements of the aldehyde given off by the ripening pears to parallel the estimation of residual aldehyde present in the tissues. A study of this character should present a clearer conception of the relations existing between the above-mentioned factors and their influence upon acetaldehyde economy in pear tissues. Methods necessary to carry out this type of investigation are now being developed.

LOSS OF ALDEHYDE IN BROWNEED TISSUES

It was previously reported (1) that after complete browning of the cells occurred, the concentration of acetaldehyde in the browned tissues showed a decided falling off, and it was concluded that the injured or dead cells were either incapable of producing acetaldehyde or unable to retain it.

This loss of aldehyde from the browned tissues was further demonstrated in the present study. Examination of the data shows a marked decrease of aldehyde following progressive injury and apparent death of the cells. The critical stage at which this loss of aldehyde becomes evident seems to vary with the maturity of the pears at the time of harvest. In the early picked lots, aldehyde began to decrease soon after the appearance of breakdown. However, in the late picked pears, aldehyde production apparently did not cease until some time after breakdown was first observed, and during this period injury continued until complete breakdown of all the tissues occurred.

CARBON DIOXIDE AND OXYGEN IN THE INTERCELLULAR ATMOSPHERE OF PEARS

Since acetaldehyde production appears to be a product of the respiratory mechanism, a measure of other substances of like origin is desirable for a broader interpretation of the process. Plant physiologists have long used the output of carbon dioxide as an index of respiratory activity; in fact, some abnormal conditions existing in fruit, including pear breakdown, have been attributed to a killing of the cells as a result of an increased concentration of carbon dioxide or of an insufficient oxygen supply.

The processes associated with the life of a cell are no doubt governed to a large extent by the gaseous medium surrounding it. Therefore, a measure of the carbon dioxide and oxygen in immediate contact with the tissues may be of more value in this study than the gross output.

In Tables 1, 2, and 3 are to be found the percentages of carbon dioxide and oxygen present in the intercellular atmosphere of pears under the conditions described. In general, with the ripening of the pears at 22°-24° C., there occurred an increase of carbon dioxide which was accompanied by a decrease of oxygen. The initial percentages of carbon dioxide in the early picked fruit were lower than

those in the later picked fruit, and the rate of accumulation was less rapid. This held true not only for the immediately ripened fruit, but also for pears ripened at 22°-24° C. after one and two months in cold storage. This is in agreement with the findings of Magness and Ballard (5) in their measurements of the carbon dioxide given off by Bartlett pears following both early and late picking.

In comparing fruits picked on the same date, it was found that those held in cold storage before ripening at 22°-24° C. contained higher percentages of carbon dioxide and the rate of accumulation accelerated more rapidly than those placed at 22°-24° C. immediately after harvesting.

The highest percentage of carbon dioxide in a given lot of fruit was usually found in the tissues some time prior to the first appearance of core breakdown. This is in contrast with the aldehyde content, which was found to be highest at the time browning of the tissues occurred. This fact may be of importance in establishing a relation of carbon dioxide concentration within the tissues to the occurrence of breakdown, but, since the highest concentrations of carbon dioxide occur in advance of the appearance of breakdown, it is not likely that this is the active causal agent in producing breakdown in pears. Moreover, in Table 3, it will be noticed that the pears picked on September 12 showed slight breakdown when removed from storage. The acetaldehyde concentration of these fruits, analyzed immediately upon removal, was comparatively high, whereas the carbon dioxide percentage was as low as that of the normal fruit of the earlier pickings. These data, therefore, make it improbable that carbon dioxide alone is the direct agent responsible for the breaking down of the tissues. However, from the work of Thomas (8) it is known that the concentration of carbon dioxide in apple tissues exerts a decided influence upon the respiratory mechanism. He found that carbon dioxide in the presence of abundant oxygen may cause the respiration of apple cells to be changed to a zymasic or anaerobic type and that acetaldehyde is a product of this type of anaerobic respiration.

Examination of the oxygen values indicates that no true carbon dioxide-oxygen ratio exists in the intercellular atmosphere as ripening progresses. In some cases high percentages of oxygen can be found in the tissues, though the carbon dioxide in the ripe pears ranged from 20 to more than 30 per cent. This strongly indicates that the respiration of Bartlett pears may be in part intramolecular in nature.

The evidence secured in this investigation indicates that the rôle of carbon dioxide and oxygen in the production of core breakdown in Bartlett pears is in the establishment of a critical environment necessary for the production of acetaldehyde.

SUMMARY

A study has been made of the acetaldehyde content of Bartlett pears, its relation to core breakdown as influenced by the time of harvesting, and the carbon dioxide and oxygen concentrations within the tissues.

It was found that the length of the period elapsing from the time the pears were picked until core breakdown occurred depended upon

the maturity of the fruit when harvested. Pears picked at an advanced stage of maturity broke down at a more rapid rate than the earlier picked fruit.

No trace of acetaldehyde could be found in pears analyzed immediately after removal from the tree, regardless of the stage of maturity. After two days in the ripening room at 22°-24° C. the tissues contained distinct quantities of aldehyde, and from this time on there was an increase, the highest concentration being reached about the time breakdown occurred.

It has been demonstrated that acetaldehyde accumulation was more rapid in the late picked pears than in the early picked and less mature fruits.

The initial percentages of carbon dioxide in the intercellular gases of the early picked fruit were lower than those in the late picked fruit, and the rate of accumulation was less rapid. The maximum carbon dioxide content of the intercellular gas was usually present some time before the first visible indication of breakdown.

In general, with an increase of carbon dioxide there was a corresponding decrease of oxygen as the pears ripened. However, in some cases fairly high percentages of oxygen accompanied high percentages of carbon dioxide. This indicates that the respiration of Bartlett pears may be in part intramolecular, which is in agreement with suggestions made by Magness and Ballard (5).

The results of this study indicate that the relation of carbon dioxide to the production of core breakdown in Bartlett pears is in the establishment of optimum conditions for the production of acetaldehyde.

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STARCHLIKE RADIATE CRYSTALS PRODUCED BY BACTERIUM MARGINATUM IN STARCH MEDIA¹

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INTRODUCTION

Bacterium marginatum L. McC.² is a plant pathogene rather widely distributed in the United States and Canada, and it has also been reported from Europe. It causes a destructive disease on both the foliage and the corms of gladioli. This organism when grown on potato-dextrose agar or other suitable media produces in great abundance beautiful spherocrystals (fig. 1) which become blue when treated with iodine solution. In most cultures the crystals are 15 to 35 μ in diameter, but some of 140 μ have been found. They are apparently composed of needlelike parts radiating from a central point. Often there are two centers, and a double form results. (Fig. 1, B.) When broken by pressure on the cover glass they split into irregular triangular and needlelike parts.

In spite of the numerous radiating lines, the crystals are transparent. By polarized light they are faintly luminous, and the larger forms show a dark cross with the lines intersecting at right angles in the center. Some crystals show, in addition to the cross, several alternate light and dark concentric circles extending from near the center to the circumference, others have irregular or broken concentric light and dark markings, and still others show no trace of such markings. These concentric circles became invisible when a selenite plate was placed between the Nicols. Other crystals of normal appearance, but developed in a different medium, showed the dark cross less distinctly and none of the concentric marking. With a selenite plate these crystals showed two yellow and two blue-violet sectors. Still other crystals examined by C. L. Alsberg, of the Food Research Institute at Stanford University, Calif., showed "a very faint cross" and with a selenite plate between the Nicols "an alternate blue and yellow concentric-circle arrangement."

Schardinger^{3 4} discovered a thermophilic bacillus, *B. macerans*, which converted a considerable amount of the starch in a culture medium into a crystalline material which he first (1903) designated as "Krystallisierte Dextrine" and later (1909) as "Krystallisierte Amylodextrin." Other workers have studied these substances, which are now generally known as crystalline amyloses.

¹ Received for publication Feb. 28, 1929; issued October, 1929.

² McCULLOCH, L. A LEAF AND CORM DISEASE OF GLADIOLI CAUSED BY BACTERIUM MARGINATUM. Jour. Agr. Research 29: 159-177, illus. 1924.

³ SCHARDINGER, F. ÜBER THERMOPHILE BAKTERIEN AUS VERSCHIEDENEN SPEISEN UND MILCH, SOWIE ÜBER EINIGE UNSETZUNGSPRODUKTE DERSELBEN IN KOHLENHYDRATHALTIGEN NÄHRLÖSUNGEN DARUNTER KRSTALLISIERTER POLYSACCHARIDE (DEXTRINE) AUS STÄRKE. Ztschr. Untersuch. Nahr. u. Genussmfl. 6: 874. 1903.

⁴ UEBER DIE BILDUNG KRSTALLISIRTER, FEHLINGSCHES LÖSUNG NICHT REDUZIERENDER KÖRPER (POLYSACCHARIDE) AUS STÄRKE DURCH MIKROBIELLE TÄTIGKEIT. [VORLÄUFIGE MITTHEILUNG AUS DER K. K. ALLGEMEINEN UNTERSUCHUNGSANSTALT FÜR LEBENSMITTEL IN WIEN.]. Centbl. Bakt. [etc.] (11) 22: 99-103, illus. 1909.

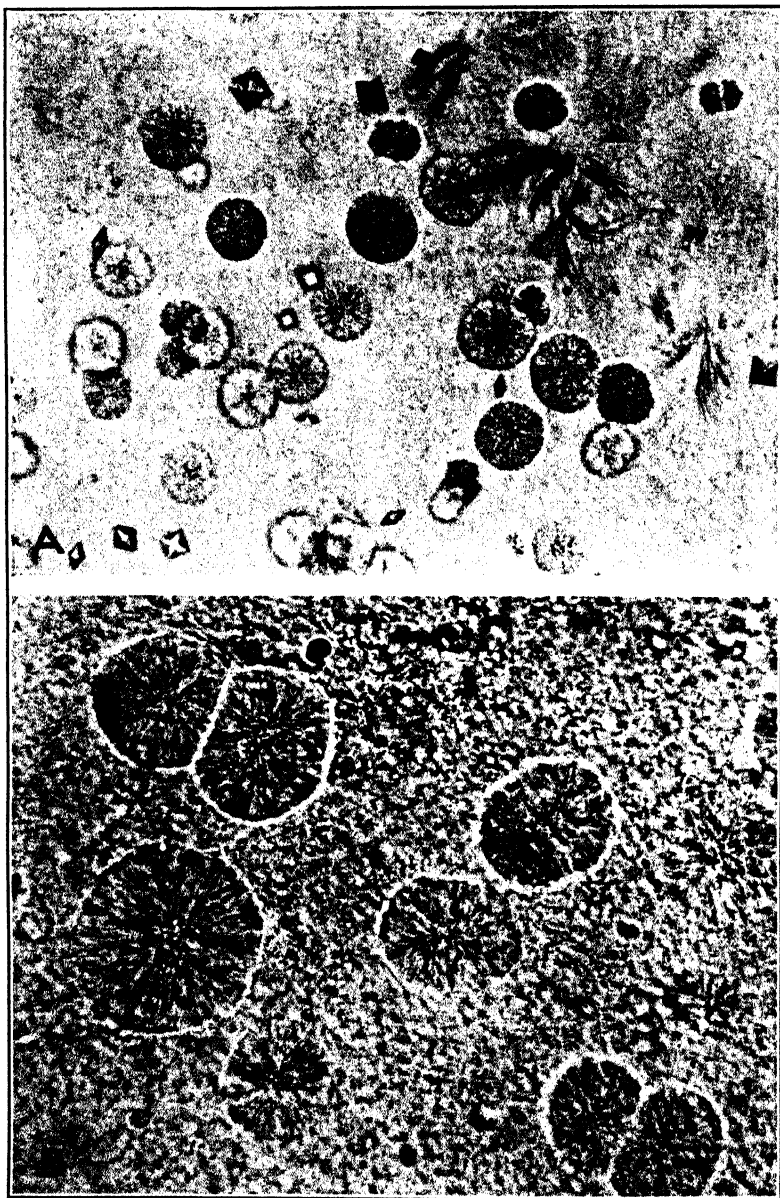


FIGURE 1.—A, Spherocrystals and also several rectangular crystals of calcium oxalate and tufts of slender, flexible crystals(?). $\times 250$. B, Spherocrystals. $\times 500$

The crystalline substances produced by *Bacillus macerans*, as described by Schardinger, are in form unlike those produced by *Bacterium marginatum*. However, Schardinger seems to have made microscopic examinations of his material only after various processes of filtering, neutralizing, and evaporation, which may have changed the form of the crystals. The morphological and cultural characters of *B. macerans* as given by Schardinger are very unlike those of *Bact. marginatum*.

Van de Sande-Bakhuyzen⁵ pointed out that starch grains are built up of radiating needles. In a later paper⁶ he demonstrated that under certain conditions spherocrystals are formed in an amylose solution which was obtained by grinding and then washing and centrifuging starch grains in cold water. The crystals so obtained are composed of several or numerous radiating needles. An alcoholic solution of iodine colors them brown to purple.

By heating a 10 per cent starch paste and then cooling it slowly, Beijerinck⁷ obtained a crystalline deposit consisting of fine needles either isolated or in groups of various shapes. These he considered crystallized starch on account of their behavior toward diastase and chemical reagents.

CULTURE MEDIA

The crystals formed by *Bacterium marginatum* have been found only in culture media containing starch and a sugar or an alcohol. Potato agar with 2 per cent of dextrose⁸ has been most commonly used, but a 2 per cent solution of pure starch in a synthetic solution has been successfully substituted for the potato broth. Saccharose, lactose, maltose, galactose, mannitol, and glycerol have each been successfully substituted for the dextrose.

The crystals form in either solid or liquid media. In solid media they are found in the medium, not in the layer of bacterial growth. In liquid media most of the crystals are found in the thick, almost viscid surface growth.

The crystals develop in the cultures during or just following an acid fermentation (without development of gas) and destruction of the sugar. Under favorable conditions the crystal formation, starting in the medium immediately adjoining the bacterial growth, proceeds into all parts. Crystals have been found in cultures on the third day after inoculation, and by the sixth or seventh day they are usually abundant in the upper half of the medium. They have been found in agar tubes 45 mm. below the bacterial growth and in plates 70 mm. from the nearest colony. They are apparently equally numerous (500 to 800 to the square millimeter of agar) in all parts of the culture. In agar cultures the crystals retain their structure and characteristic reaction with iodine solution in old (some 5 years old) dry cultures.

As the acid fermentation proceeds the slightly cloudy, opalescent potato-dextrose agar becomes more transparent. A cloudy line (due to closely packed, tiny, irregular crystals) usually divides the trans-

⁵ VAN DE SANDE-BAKHUYZEN, H. L. THE STRUCTURE OF STARCH GRAINS OF WHEAT GROWN UNDER CONSTANT CONDITIONS. Soc. Expt. Biol. and Med. Proc. 23: 302-305. 1926.

⁶ ——— CRYSTALLIZATION OF STARCH. Soc. Expt. Biol. and Med. Proc. 23: 506-507. 1926.

⁷ BEIJERINCK, M. W. "CRYSTALLIZED STARCH." K. Akad. Wetensch. Amsterdam, Proc. Sec. Sci. 18: 305-309, illus. 1916.

⁸ Potato, 500 gm., sliced and steamed, in 1,000 c. c. distilled water, strained; 1½ per cent agar added; steamed again, filtered; 2 per cent dextrose added; tubed, and sterilized by autoclaving.

parent fermented part from the unfermented, the line advancing as the fermentation proceeds. (Fig. 2, A and B.) The spherocrystals are abundant in the cleared, fermented area; they are occasionally found in or just beyond the cloudy line, but are entirely lacking in the unfermented part. Tests made with Fehling's solution at the close of the fermentation period show that all the sugar has disappeared, while the starch content seems unchanged. The tests for starch have been made in only a crude way by adding iodine solution and comparing with checks. In most cultures there seems to be no difference in the starch reaction between cultures and checks. In others there is apparently less starch in the cultures. More exact methods will be necessary to decide how much starch, if any, has been changed. If peptone is added to the culture medium the production of the crystals is apparently hindered, but not prevented, and the sugar is less completely destroyed.

The exact conditions and media for the best production of these crystals have not yet been determined. The potato-dextrose agar is not always suitable. Sometimes there are variations in parallel cultures of the same strain of the bacteria grown on the same medium. One tube may have numerous crystals and others few or none. A few cultures have been observed with crystals in only certain areas. Sometimes an increased amount of sugar favored and at other times seemed to hinder crystal production.

The natural pH value of the potato-dextrose medium varies from 5.2 to 6.6. By adjustment, values from 4.8 to 7.9 have been made. While the bacteria grew on all of these, the values from 5.2 to 6.6 appeared most favorable. Regardless of the original pH of the medium, the first reaction to the bacterial growth is acid. The peak of the acid reaction is 4.2 to 4.4, after which there is a change toward the alkaline, sometimes reaching 8.4.

The best results up to the present both for numbers and size of the crystals were secured with a soft agar medium of pH 5.4 (potassium biphosphate, 0.2 gm.; asparagine, 1.5 gm.; dextrose, 5 gm.; galactose, 5 gm.; potato starch, 10 gm.; water, 500 c. c.; agar, 2.5 gm.). A liquid medium of pH 5.2 (potassium biphosphate, 0.2 gm.; potassium chloride, 0.05 gm.; magnesium sulphate, 0.05 gm.; calcium sulphate, 0.01 gm.; asparagine, 3 gm.; dextrose, 20 gm.; corn starch, 20 gm.; trace of iron; water, 1,000 c. c.) induced a heavy surface growth of bacteria, and numerous crystals were present three weeks after inoculation. Three months later the crystals were comparatively scarce and irregular in shape but still reacted typically with iodine solution. Another agar of pH 6.6 (potassium nitrate, 0.04 gm.; dextrose, 4 gm.; soluble starch, 2 gm.; agar, 2 gm.; water, 200 c. c.) was not favorable for the bacterial growth, but some of the starchlike crystals were produced.

Cultures were grown at temperatures ranging from 25° to 30° C.

It occasionally happens that, due to some unknown condition, the bacterial activity is limited in media supposed to be favorable. In such cases little or no fermentation occurs and no starchlike crystals develop; or some fermentation may occur without the formation of crystals.

A rough type of colony that occasionally appears in plate cultures of the normally smooth-surfaced colonies of *Bacterium marginatum* also produces the starchlike crystals.

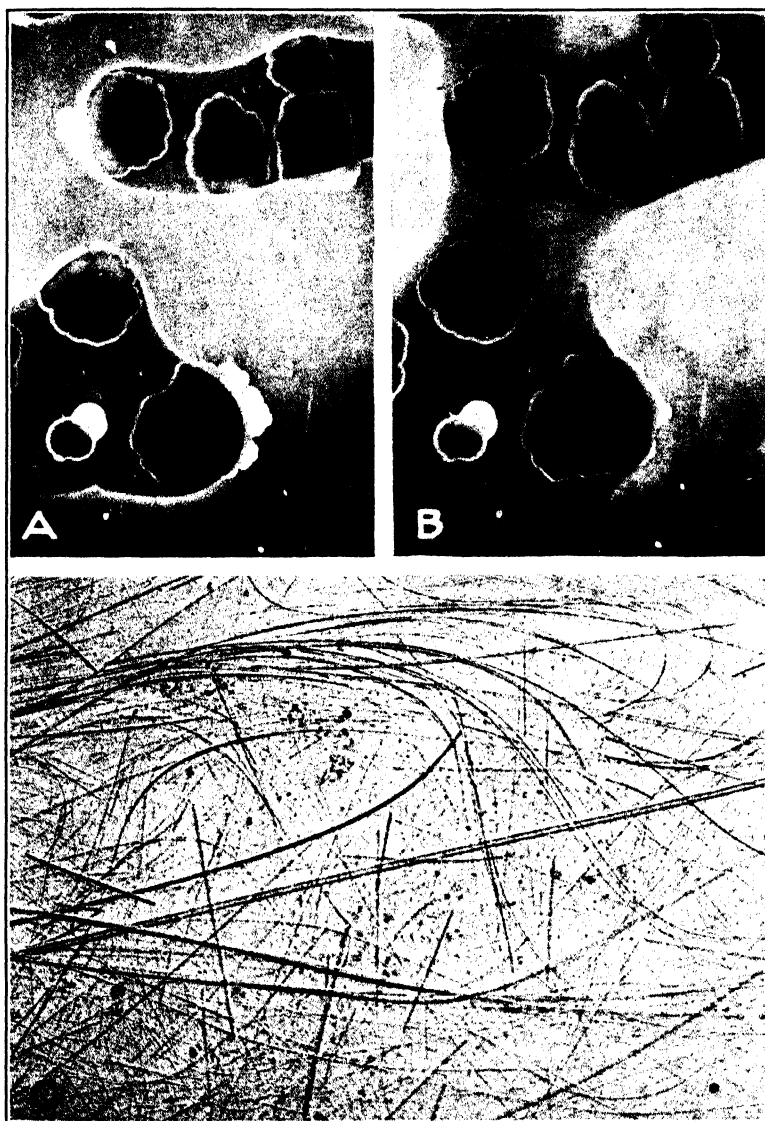


FIGURE 2.—A, Colonies of *Bacterium marginatum* in potato-dextrose agar, showing the cleared area surrounding the colonies. $\times 1$. B, Same colonies 24 hours later, showing increased size of the cleared area. $\times 1$. C, Long, flexible crystals(?) from potato-dextrose agar cultures of *Bact. marginatum*. $\times 250$

All of the 20 or more strains of *Bacterium marginatum* used in the experiments have produced these starchlike crystals, but some strains have more readily and regularly than others produced crystals of good size. Some strains which formerly produced the crystals now fail to do so.

A number of other species of bacteria have been grown in parallel cultures with *Bacterium marginatum*, but none of them produced the type of crystal found so abundantly in the *Bacterium marginatum* cultures.

TEMPERATURE AND CHEMICAL TESTS

Because these crystals react like starch with iodine solution and are produced only in the presence of starch, it seems probable that they are some form of starch; but as no way has yet been found to separate the crystals from the agar of solid cultures or from the bacterial slime of liquid cultures, their exact chemical nature is unknown. In the following tests, agar containing typical crystals was crushed to a paste and the whole mass treated together.

The crystals do not dissolve in cold water, but after several days in it they become very transparent and sometimes invisible until they are stained with iodine. In water at 90° to 100° C. they dissolve quickly, but they withstand water at 80° for at least one hour. Alcohol, both ethyl and amyl, 50 to 95 per cent, has no effect on them in three hours. There is no effect from ether, chloroform, acetone, saturated solution of sodium phosphate, acetic acid (1, 10, 50, and 100 per cent), ammonia (concentrated), or 20 per cent picric acid. Alcohol (90 per cent) plus a small amount of sulphuric acid has no effect in two hours, but in 50 per cent sulphuric acid in alcohol the crystals fade away, without gas formation, in one minute or less. In iodine solution the crystals become pale blue to black, depending on the strength of the solution and also on the permeability of the surrounding media. Millon's reagent, cold for 15 minutes, has no effect, but when heated slightly the crystals dissolve without any trace of color reaction. In 5 per cent sodium hydroxide the crystals dissolve. After 20 hours in hydrogen dioxide the crystals are cracked and larger. After 12 hours in a strong solution of chloral hydrate there is no visible change in the structure of the crystals. In a 5 per cent diastase solution, at 35° to 37° C., the crystals disappear in one and one-half hours or less.

ASSOCIATED CRYSTALS

While the starchlike crystal is the most interesting crystal formed in cultures of *Bacterium marginatum*, there are several other types. Most frequently seen are those of calcium oxalate (fig. 1, A), either as small granules or as large 8-sided forms up to 75 μ in size. These occur not only in media suitable for starchlike crystals but also in beef agar, beef bouillon, plain potato broth, and 2 per cent peptone solution.

In potato-dextrose agar cultures there are found some extremely long and slender hyaline forms which were at first supposed to be crystals. There is one record of some that polarized light. It is more likely that they are some sort of gummy substance. The diameter varies from 0.5 to 1.5 μ , usually uniform throughout the

whole length, which is 10 to 700 μ . In cross section they are not round, but square or rectangular, the side walls being definitely at right angles. The longer forms are very flexible. They will bend almost double but break over a sharp angle. Often a number of these slender forms seem to originate at a common center. (Fig. 1, A.) Pressure on the cover glass usually causes them to flatten, some of them to 10 or more times their original width, and occasionally irregular enlargements form at the ends. Parallel close-lying threads sometimes coalesce, and eventually with continued pressure these crystals (?) disappear, seeming to melt and leave no trace. No water or other mounting medium was used. In cultures 2 to 3 months old these crystals (?) were still flexible, but instead of flattening or dissolving under pressure, they broke into short pieces.

Besides the preceding types, other less characteristic crystals are found, mostly in old cultures. These are irregularly spherical forms resembling hailstones, frost crystals, and rough-branched coral. Hollow brittle spheres 1 to 1.5 mm. in diameter occasionally appear in milk cultures. Their walls seem composed of minute crystals and some amorphous material. They do not dissolve in water or in 10 per cent potassium hydroxide. Alcohol, chloroform, ether, ammonia, acetone, and xylol have no effect on them, but they dissolve in dilute acetic, hydrochloric, or sulphuric acid.

SUMMARY

This paper describes a starchlike crystal which forms in cultures of *Bacterium marginatum* when starch is a constituent of the medium. These spherocrystals, up to 140 μ in diameter, are produced in great numbers. They are composed of needlelike or threadlike parts radiating from the center. They become blue when treated with iodine solution, and by polarized light the larger crystals show dark cross marks.

The chemical nature of these crystals has not been determined, because as yet no way has been found to separate them from the culture medium.

INFLUENCE OF BACTERIOPHAGE ON BACTERIUM TUMEFACIENS, AND SOME POTENTIAL STUDIES OF FILTRATES¹

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INTRODUCTION

While making potentiometer studies of the juices of 50 different species of healthy plants as compared with the juices of tumors produced on the plants by *Bacterium tumefaciens* Sm. and Town., the junior writer was impressed by the frequent reversal of the relative magnitudes of the pH values in the various juices after they had oxidized from one to five days. It was observed that whereas the juice of the normal sugar beet immediately after crushing might be pH 5.9 and that of the crushed tumor 6.3, in two days the juice of the normal sugar beet would be 6.2 and that of the tumor 4.8. It occurred to her that this oxidized tumor juice might influence the organism producing the tumor and that by growing the organism in a suitable medium, along with the oxidized tumor juice which had reversed its pH relative to the pH of the normal juice, there might be obtained a culture that had lost its power to infect.

Two cultures of *Bacterium tumefaciens* were accordingly exposed to different dilutions of the oxidized juice from crushed Ricinus tumors, and after the cultures had been incubated for a few days young Ricinus plants were inoculated with them. At the same time other Ricinus plants were inoculated with control cultures. Although neither treated culture became inactivated as was expected, one produced more rapidly forming and larger tumors and the other more slowly forming and smaller tumors than the controls. Recognizing that something besides oxidation with accompanying pH change had entered into these results, and believing that it was the principle of bacteriophagy, the junior writer began the work which developed into the joint investigation here reported.

REVIEW OF LITERATURE

The isolation from diseased plants of a lytic and inhibiting principle, called by D'Hérelle (3)² the bacteriophage, has not been carried on so extensively by plant investigators as by animal research workers. The earliest plant investigators who succeeded in isolating lytic principles from nodules of leguminous plants, from roots and stems of legumes, and from garden and field soil were Gerretsen, Gryns, Sack, and Söhngen (2). Mallmann and Hemstreet (7) isolated an inhibiting substance from a rotted cabbage, but did not demonstrate actual lysis. Coons and Kotila (1) isolated from rotted carrot, river water,

¹ Received for publication Mar. 13, 1929; issued October, 1929.

² Reference is made by number (italic) to "Literature cited," p. 529.

and soil the bacteriophage which produced inhibition of growth of various bacteria when used in high dilutions and definite lysis when used in stronger concentrations.

In Russia, Israilevsky (Izrailevsky) (5, 6) demonstrated the bacteriophage in plant tumors produced by *Bacterium tumefaciens*. From nine different isolations from tumors on sugar beets he succeeded in getting two strains which were dissolved by the bacteriophage. In his later work (6) he studied the physical-chemical nature of the normal-plant and tumor juice along with that of *Bact. tumefaciens* in culture and concluded that the disappearance of *Bact. tumefaciens* from plant galls must be attributed to the action of a bacteriophage and not to the formation of acid in the galls. He also treated roots, stems, and seeds with the bacteriophage before inoculating them with *Bact. tumefaciens* and found that the bacteriophage reduced appreciably the percentage of infection. He stated, however, that a great many more experiments were necessary before the prophylactic action of the bacteriophage is unquestionably established.

PRELIMINARY EXPERIMENTS

In the preliminary plant inoculations made by the junior writer tumors of two distinct types were produced—one more rapidly growing and larger in size than the control tumors, and the other slow in appearing and smaller in size than the controls. The cultures were made as follows:

Good-sized *Ricinus* tumors were crushed well and the juice was extracted and allowed to oxidize three days, in which time the pH changed from 5.6 to 5.0. The juice was filtered through paper, and dilutions in beef bouillon of pH 7.0 were carried to about 1 : 1,400 and 1 : 28,000. The tubes were seeded with a 24-hour-old culture of *Bacterium tumefaciens* (hop strain) and left to grow two days. From these dilutions transfers were made to potato-dextrose agar, and *Ricinus* plants were inoculated with them two days later. Other *Ricinus* plants of the same age were inoculated with a potato-dextrose agar control culture 2 days old.

In 10 days the inoculations with the 1 : 1,400 dilution culture showed no indication of outgrowths, those with the control cultures had produced swellings 2 to 3 mm. in diameter, and those with the 1 : 28,000 dilution culture had produced little tumors 5 to 6 mm. in diameter. In 19 days very tiny swellings were showing from the inoculations made with the 1 : 1,400 dilution (fig. 1, A), whereas the inoculations made with the 1 : 28,000 dilution (fig. 1, B) had produced tumors twice as large as the controls, (fig. 1, C).

This comparative rate of growth kept up for over a month, the differences being quite marked. In one and one-half months the rapidly growing tumors had reached their limit, 6 cm. in diameter. This was twice the size of the controls. The retarded tumors began to grow rapidly one month after inoculation, but in two months after inoculation the largest of them was only 1.5 cm. in diameter. Very little development took place after that.

TECHNIC

Filtrates of *Ricinus* tumors and of sugar-beet tumors produced with the hop strain of *Bacterium tumefaciens* were added to fresh beef-bouillon cultures of the hop strain, the 2, 10, and 30 drop method



FIGURE 1.—Accelerated and retarded tumors on *Ricinus*. Plants inoculated January 21, 1927; photographed after 19 days. All natural size. A, Retarded and undersized tumors produced by *Bacterium tumefaciens* (hop strain) plus a low dilution of tumor juice; B, accelerated and oversized tumors produced by *Bact. tumefaciens* plus a higher dilution of tumor juice; C, normal and regular-sized tumors produced by a control (normal) culture of *Bact. tumefaciens*.

of D'Hérelle (3) being used. These drop cultures were watched for inhibition and lysis. Several 30-drop cultures were usually made, one of which was used to make slant cultures and poured plates for the study of bacteriophage plaques. One of the 30-drop filtrate tubes had a little heavier seeding of the organism than the others, which received only a loop transfer from the young culture. The transfers to the beef bouillon were made from an 18 to 24 hour beef-bouillon culture faintly clouded or from an agar slant of the same age suspended in beef bouillon. The former was found more satisfactory, as the hop strain produces bacterial clumps, a feature not wanted in this type of work. Infusion-beef agar, pH 7.0 to 7.2, was found satisfactory for demonstrating the plaques on the plates. Beef-bouillon media of pH 6.5 to 8.3 were tried for demonstrating inhibition in the seeded filtrate cultures, but those of pH 6.7 to 6.9 were found more satisfactory. Incubation was at temperatures of 22° to 28° C.

Plates were poured from the filtrates to test their sterility, and if they proved not to be sterile this fact was noted.

When plates were poured from the seeded filtrate for the study of plaques, plates of the control cultures were also poured for comparison. Slant cultures were often made from the 2 and 10 drop seeded filtrate cultures as well as from the 30-drop ones. To seed the slants and plates, drops of cultures were carried to them in sterile pipettes, although a sterile cotton swab was sometimes used for this purpose. The seeded poured plate was found the most satisfactory of all methods to demonstrate the plaques.

Except in the case of the first experiment which demonstrated the acceleration and retardation of the growth of tumors, the juices were filtered. Chamberland L3 filters were used after the juice had first been put through filter paper or centrifuged. If one of the 30-drop seeded filtrate cultures showed inhibition or slight growth in 24 hours it was refiltered and the organism exposed again to the new filtrate. These passages of the organism with the filtrate through successive trials were carried along to increase the potency of the inhibiting substance present, in the hope that lysis would be attained. Sometimes only one and at other times several filterings and seedings were made in eight hours.

Dilutions of the tumor filtrates from 1:10 to 1:10,000,000,000 in tubes with beef bouillon seeded with the organism were also used for the study of inhibition and lysis. Control cultures were always held in the same racks for comparison.

Filtrates of normal *Ricinus* juice, of carrots rotted with *Bacillus carotovorus*, of normal carrots, and of sewage were also tested with the organism by both the drop and the high-dilution methods.

The strains of *Bacterium tumefaciens* tested were hop, peach, and daisy.

Plants of *Ricinus communis* (castor-oil plant) were used for the inoculations because they grow fast in the greenhouse and produce tumors quickly. The hop strain of *Bacterium tumefaciens*, which was used for most of the work, produces 100 per cent infection on *Ricinus*.

PHYSICAL-CHEMICAL ASPECTS OF THE FILTRATES

The study of *Bacterium tumefaciens* in culture and experiments on plants with chemical substances produced by *Bact. tumefaciens*, together with experiments limiting the intake of oxygen and so compelling the cells to manufacture the stimulus which leads to the development of hyperplasias, led Smith (9) to state that—

All tumors, so far as they are due to parasites, must be assumed to be due to the chemical-physical action of the by-products of the metabolism of these parasites, just as most communicable diseases are due not to the parasites themselves, but to their toxins. * * * Diverse as are the tumors of plants due to parasites, I think that the fundamental chemical-physical phenomena underlying them are much alike, and that the differences we see, when these differences are not due wholly to varying tissue reactions, must result simply from variations in volume, and direction and continuity of the chemical-physical stimulus.

Since these statements were published, much has been added to confirm Smith's conclusions by the more direct pH, total-acid, and oxidation-potential measurements and dilution experiments on the juices of normal and tumor tissues. The striking facts revealed by these later studies are: (1) The pH of the freshly extracted juice of tumor tissue was always higher or the reaction more alkaline than that of the normal juice; (2) the titratable-acid content was greater in the tumor than in the normal juice; (3) the oxidation potential was always greater (more negative) in the freshly extracted tumor juice than in the normal juice—that is, the electrical charge carried by the gold electrode in the tumor juice was always negative (—) to that in the normal juice; (4) the electrical charges (accompanied always by a change in pH) on the gold electrodes were exactly reversed in the normal and tumor-tissue juices upon further oxidation; and (5) the change in pH produced by the growth of the organism (*Bacterium tumefaciens*) in beef bouillon cultures and the change in pH produced by *Bacillus carotovorus* when inoculated into fresh carrots progressed in the same direction (increase) as the change of pH of the juice of the normal tissue on further oxidation, rather than in the direction (decrease) of the pH changes of the juice of the tumor tissue.

The juices of the tumor are always more colloidal than the juices of normal tissue, as evidenced by the degree of filterability. The influencing factor is not precipitated by centrifugation and is active in high dilutions as an accelerator of *Bacterium tumefaciens* and in low dilutions as an inhibitory factor.

Since the substance in the plant filtrates which in the presence of molecular oxygen are apparently associated with oxidation and reduction phenomena causing changes in the pH and their oxidation potentials (with gold electrodes) behaved the same in all plants studied, and were consistently linked with the normal and tumor-tissue juices, it was thought that such potential determinations might give evidence when bacteriophage plaques could be expected on poured plates. That there was some justification for this belief is brought out by a study of Table 1 and the poured-plate and plant-inoculation experiments given later.

TABLE 1.—*Titratable-acid, pH, and oxidation-potential measurements^a of different filtrates and the plaques on poured agar plates resulting from treating the cultures of Bacterium tumefaciens (hop strain) with them*

Filtrates and cultures tested	Freshly extracted filtrates and young cultures				Oxidized filtrates and old cultures		Plaques
	Initial pH	N/1 alkali required to increase pH to 8.2	Oxidation potential differences	Charge (plus or minus)	Initial pH	Charge (plus or minus)	
Ricinus with mature tumor:		<i>C. c.</i>	<i>Millirots</i>				
Tumor	5.5	22.5	125.6	—	4.9	+	Present.
Normal	5.4	16		+	6.8	—	
Ricinus with rotted tumor:							
Tumor	6.8		103.0	—	7.1	—	Absent.
Normal	5.4			+	8.2	—	
Ricinus with dried tumor plus water:							
Tumor	6.8			—	6.8	—	Very few present.
Normal	5.5			+	8.0	—	
Sugar beet with mature tumor:							
Tumor	6.2	23.5	125.0	—	5.0	+	Present.
Normal	5.9	18		+	6.4	—	
Sugar beet with older, mature tumor:							
Tumor	6.8	25	143.2	—	5.1	+	Do.
Normal	6.3	19.5		+	6.8	—	
Carrot:							
Normal, fresh and young	6.8						
13-day-old normal, young					7.1		
Inoculated 70 days with <i>Bacillus carotovorus</i> .					9.6		
<i>Bacterium tumefaciens</i> :							
Plus juice of carrot infected with <i>B. carotovorus</i> ; culture 1 day old.	^b 7.1						Many present
Plus juice of carrot infected with <i>B. carotovorus</i> ; culture 4 days old.					6.6		
24-hour-old normal culture	6.7						Present.
1-month-old normal culture					8.2		Absent.

^a The following procedure was suggested by S. F. Acree of the Bureau of Standards: Two batteries, consisting each of a saturated calomel electrode and a Hildebrand cell, were joined by a platinum wire, connecting the two calomel cells. Each Hildebrand cell contained a gold or a hydrogen electrode as desired. Usually 10 c. c. of the normal or tumor juice was used for each test. This equipment made it possible (1) to measure both the hydrogen-ion and the oxidation potentials against a standard calomel cell, or (2) to obtain directly the differences in these potentials shown by the normal and tumor extracts. The latter method was used generally with occasional checks of each sample against the calomel electrode with a type K Leeds & Northrup potentiometer. The estimation of potentials was made on expressed juice of normal and tumor tissue. The same volume of normal and tumor juice from the same plant was used for comparison in each experiment. The same environmental conditions as to temperature, gases, technic, etc., made the results more accurate as they are dependent on the simultaneous handling of both samples.

^b This culture was split and tested 4 days later, but no plates were poured on the fourth day.

It is shown in Table 1 that the freshly extracted juice from *Ricinus* and sugar beet gave pH values of 5.4 to 6.3 and carried a positive (+) charge when compared to the juice of the tumor; the same juice oxidized from two to five days gave pH values progressing in the alkaline direction accompanied by a minus (—) charge. The opposite relationship of values, both as to pH and to charge carried, to those of the normal juice was obtained on the freshly extracted and oxidized tumor juice. The freshly extracted juice of a mature tumor was more alkaline than the normal juice in its initial pH values and carried a minus (—) charge, but upon oxidation for two to five days the pH values became distinctly more acid with an accompanying change of electrical charge (+). *Bacterium tumefaciens* treated with this filtrate showed numerous plaques on the poured plates. In the case of the rotted tumor juice the pH value proceeded in an alkaline direction, and in that of the dried tumor juice plus water the value remained about

stationary. It is a striking fact that from the last two filtrates, where the pH progressed in the alkaline direction or remained stationary, few or no plaques were found on the poured plates. The total acid content was found to be greater in the freshly extracted tumor juice than in the normal juice; also a definite oxidation-potential value in favor of the freshly extracted tumor juice above that of the normal juice was registered. The reversal of pH ceased at about pH 5.1 to 4.9 in the juices of the tumors from *Ricinus* and sugar beet.

The freshly extracted juice of young carrots gave pH values of 6.8, whereas the same juice left standing 13 days gave pH 7.1. A *Bacterium tumefaciens* culture treated with the fresh filtrate of normal carrot produced plaques. Juice of carrots 70 days after inoculation with *Bacillus carotovorus* gave pH 9.6. The pH direction taken by carrot inoculated with *B. carotovorus* is the same as that taken by *Bact. tumefaciens* in beef-infusion medium and that of the normal-carrot juice upon further oxidation.

A freshly seeded *Bacterium tumefaciens* culture treated with the filtrate of carrots inoculated with *Bacillus carotovorus* gave a value of pH 7.1. With this filtrate the plaques on poured plates were more numerous than with any of the other filtrates. This same culture held four days gave pH 6.6. Here again the pH is in the more acid direction when compared with the values of the juice from carrot inoculated with *B. carotovorus* (pH 9.6) and *Bact. tumefaciens* culture 1 day old (pH 6.7) or 1 month old (pH 8.2). At two different times a 24-hour culture of *Bact. tumefaciens*, pH 6.7, gave good plaques on poured plates. These were exceptions rather than the rule. For the range of growth of *Bact. tumefaciens* in beef-infusion medium an earlier paper (8) may be consulted.

Three *Ricinus* plants were inoculated with each of the tenfold dilution cultures when the cultures were two days old, and three other *Ricinus* plants were inoculated with a control culture of the same age. In nine days many of the little tumors resulting from the dilution inoculations were growing faster than the control tumors. In less than a month the tumors forming on 17 of the plants inoculated with the dilution culture were larger than the control-culture tumors, 8 were of the same size, and only 5 were slightly smaller. All of the sixth-dilution (1:1,000,000) inoculations produced smaller tumors than the controls, while all the first, fifth, eighth, and two each of the seventh and tenth dilutions produced larger tumors.

To determine whether inhibition might occur if larger quantities of the same filtrate were used in the bouillon, a series of dilutions was made with 9 parts of the filtrate to 1 part of beef bouillon. To a tube of 1 c. c. of beef bouillon 9 c. c. of filtrate was added, thus making a 9:10 dilution. Ten dilutions were made as before, the last being 9:1,000,000,000. Each was seeded with a loop of a 24-hour culture of the hop strain. Heavier growth than in the controls took place in 24 hours in all cultures except the 9:10, which had none. A heavy pellicle formed in all except the 9:10. In 48 hours, however, this culture also had what appeared to be a trace of growth. Inoculations with these 11 cultures (including the control) were made the second day into each of three *Ricinus* plants.

Practically the same relationship of acceleration of tumor growth and increased size of tumors over the controls was observed in the

9:10 series as in the 1:10 series. Inoculations with the first dilution, 9:10, did not produce tumors. Table 2 gives the results of the inoculations with both series.

TABLE 2.—Results of inoculating *Ricinus* plants with the hop strain grown two days with filtrates of *Ricinus* tumors in dilutions of 1:10 to 1:10,000,000,000 and 9:10 to 9:1,000,000,000, respectively

Dilution and period between inoculation and examination	Dilutions of 1:10 to 1:10,000,000,000				Dilutions of 9:10 to 9:1,000,000,000			
	Initial pH	Diameter of tumors on—			Initial pH	Diameter of tumors on—		
		Plant No. 1	Plant No. 2	Plant No. 3		Plant No. 1	Plant No. 2	Plant No. 3
No. 1: 9 days..... 1 month.....	6.8	Mm. { 9-12 20-30	Mm. { 9-12 20-30	Mm. { 9-12 20-30	5.3	Mm. { 0 0	Mm. { 0 0	Mm. { 0 0
No. 2: 9 days..... 1 month.....		{ 9-12 30-40	0 13-20	0 9-12		{ 9-12 20-30	9-12 20-30	9-12* 20-30
No. 3: 9 days..... 1 month.....	6.8	{ 9-12 30-40	9-12 20-30	9-12 13-20	7.0	{ 9-12 20-30	9-12 20-30	6-8 9-12
No. 4: 9 days..... 1 month.....		{ 9-12 20-30	9-12 13-20	9-12 9-12		{ 9-12 13-20	0 13-20	0 9-12
No. 5: 9 days..... 1 month.....	6.9	{ 9-12 20-30	9-12 20-30	9-12 20-30	7.1	{ 9-12 20-30	9-12 9-12	6-8 9-12
No. 6: 9 days..... 1 month.....		{ 9-12 9-12	4-5 9-12	4-5 9-12		{ 9-12 9-12	0 9-12	0 9-12
No. 7: 9 days..... 1 month.....	6.9	{ 9-12 20-30	0 20-30	0 13-20	6.9	{ 9-12 9-12	9-12 9-12	6-8 20-30
No. 8: 9 days..... 1 month.....		{ 9-12 20-30	0 20-30	0 20-30		{ 9-12 20-30	9-12 20-30	0 9-12
No. 9: 9 days..... 1 month.....	7.1	{ 9-12 20-30	0 13-20	0 13-20	7.1	{ 9-12 20-30	0 20-30	6-8 9-12
No. 10: 9 days..... 1 month.....		{ 9-12 30-40	9-12 30-40	9-12 13-20		{ 9-12 20-30	0 20-30	0 20-30
Control: a 9 days..... 1 month.....	7.8	{ 4-5 13-20	0 13-20	0 13-20	7.8	{ 4-5 13-20	0 13-20	0 13-20

* Same control plants used for both dilution series.

EFFECTS OF DIFFERENT TREATMENTS ON THE HOP STRAIN

EFFECTS OF DILUTE FILTRATE OF *RICINUS* TUMORS ON PRODUCTION OF TUMOR

JUICE FILTERED AFTER 3-DAY OXIDATION

Filtrates of *Ricinus* tumor which were found to be sterile by the poured-plate method after passing through Chamberland L3 filters were studies in dilution experiments. The juice had been exposed to the air for three days before it was filtered and its pH had changed from 5.5 to 4.9. A series of tubes containing 9 c. c. of infusion-beef bouillon, pH 6.9, was treated as follows: With a sterile pipette 1 c. c. of a filtrate of a *Ricinus* tumor was added to the first tube and mixed thoroughly by shaking. This gave a dilution of 1:10. With another sterile pipette 1 c. c. of this dilution was added to the next tube, making the second a 1:100 dilution. The method was repeated, each tube was shaken and a sterile pipette was used for each of the

10 tubes of the series until a dilution of 1:10,000,000,000 was reached. The dilutions were each seeded with a loop of a 24-hour bouillon culture of the hop strain, for in a previous test a drop of the culture had been found to be too heavy.

In 18 to 24 hours growth was heavier than in the controls in all but the 1:10 dilution. In 2 days there was also growth in the 1:10 dilution.

JUICE FILTERED IMMEDIATELY

The dilution series 1:10,000,000,000 was repeated with two other filtrates (pH 5.8 and 5.5) from Ricinus tumors. As unoxidized juice was desired for this experiment, both sets were filtered, diluted in series, and seeded with the hop strain the same day the tumors were cut. No retardation of growth was observed in the tubes of either set except in the 1:10 dilutions, which lasted for only about 24 hours. Both sets were well clouded in 48 hours. The other nine dilutions showed greater growth than the controls in 24 hours.

Two Ricinus plants were inoculated with each of the 10 dilutions of the second of the unoxidized sets. Two inoculations with control cultures were also made. The tumors grew more rapidly and developed to greater size in the fifth, sixth, seventh, and eighth dilution inoculations than in the controls; the tumors in the ninth and tenth dilution inoculations were the same size as control tumors; while the tumors in the first, second, third, and fourth dilution inoculations within a little more than a month were slightly smaller than those of the controls.

From these tests it appears that tumor juices filtered and used immediately and those oxidized several days before filtering and used in high dilutions with the organism have power to produce more rapidly growing and larger tumors than the control cultures; they also have power to produce larger tumors than the controls when they are inoculated into susceptible plants.

COMPARATIVE EFFECTS OF FILTRATES OF BACTERIUM TUMEFACIENS, NORMAL RICINUS PLANTS, AND NECROSED RICINUS TUMORS

A 24-hour beef-bouillon flask culture of *Bacterium tumefaciens* was filtered, the filtrate was carried in tubes in dilutions from 1:10 to 1:10,000,000,000, and each dilution was inoculated with *Bact. tumefaciens* (hop strain) to see how the organism would react with a culture filtrate of itself added. In 24 hours there was slight growth in the controls but no growth in any of the dilutions. In 48 hours, however, there was growth in all 10 dilutions, and in many of them it was heavier than in the controls.

A filtrate of normal Ricinus plants and one of necrosed Ricinus tumors were carried each in 10-dilution tubes in the same way, 1:10 to 1:10,000,000,000, and each dilution was inoculated with the hop strain for comparison with the culture-filtrate dilutions. In 24 hours no growth had occurred in these two sets. In 48 hours, however, there was heavier growth in some of the dilutions with filtrate of the normal Ricinus plant than there was in the controls. There was growth equal to the controls in the cultures with necrosed Ricinus tumor filtrate. The pellicles were not so heavy as in the controls in the 1:10 and 1:100 dilutions of the latter.

Inoculations into young *Ricinus* plants were made when the dilution cultures were 4 days old. The plants were a little younger than is desirable for inoculating and the growing tumors killed a few of them.

Many of the culture-filtrate dilutions produced tumors more rapidly than the control cultures, the normal *Ricinus* filtrate cultures, or the necrosed tumor filtrate cultures. The tumors were also larger than those produced by the necrosed tumor filtrate inoculations, but were not very different in size from those of the normal *Ricinus* tumors. Table 3 gives details of this experiment.

EFFECTS OF REPEATED FILTERINGS WITH DIFFERENT FILTRATES

FILTRATES OF OLD AND SLIGHTLY NECROSED RICINUS TUMORS

The pH of the juice of 5-months-old *Ricinus* tumors was 6.8 before filtering and 6.2 after filtering. The pH of the juice of healthy *Ricinus* plants of the same age was 5.4.

Two, ten, and thirty drops of the 5-months-old tumor filtrate were added to fresh (hop strain) cultures in beef bouillon (pH 6.7). In 24 hours the growth was slight in the drop cultures but equal to that in the controls. Bacteriophage plaques were present on the drop plates, however, in two days. Refilterings of the drop cultures were made, and the tests with tube cultures and poured plates were carried through the third passage. There was still no inhibition in the third passage, and no bacteriophage plaques occurred in the drop-filtrate or the control plates.

Seven days later more of the 5-months-old *Ricinus* tumor juice was filtered, and *Bacterium tumefaciens* was exposed to it in the usual way. The filtrate had then changed from pH 6.8 to 7.1. A test with 2, 10, and 30 drops was made in the morning and another in the afternoon, using hop strain in beef bouillon (pH 6.7). In both sets growth of the organism was more rapid in the drop tubes than in the controls. No bacteriophage plaques were present on the drop-filtrate plates. The plates poured to test the sterility of the old tumor filtrate had contaminating but no *Bact. tumefaciens* colonies. To make a further test, the unused part of the 7-day-old tumor filtrate was filtered a second time. The filtrate was sterile, for no contaminating colonies appeared on the plates poured from it. The hop strain was again exposed to the old filtrate by the drop method to see if retardation would occur. There was none.

TABLE 3.—Results of inoculating *Ricinus* plants with *Bacterium tumefaciens* (hop strain) grown four days in 1:10 to 1:10,000,000,000 dilutions of filtrates of *Bacterium tumefaciens* (hop strain) of normal *Ricinus* plants, and of necrosed *Ricinus* tumors, respectively

Dilution and period between inoculation and examination	Filtrate of beef-bouillon culture of hop strain (pH 6.7)			Filtrate of normal <i>Ricinus</i> plants (pH 5.4)			Filtrate of necrosed <i>Ricinus</i> tumors (pH 6.8)		
	Initial pH	Diameter of tumors on—		Initial pH	Diameter of tumors on—		Initial pH	Diameter of tumors on—	
		Plant No. 1	Plant No. 2		Plant No. 1	Plant No. 2		Plant No. 1	Plant No. 2
1 to 10:		<i>Mm.</i>	<i>Mm.</i>		<i>Mm.</i>	<i>Mm.</i>		<i>Mm.</i>	<i>Mm.</i>
10 days.....	7.8	9-12	9-12	7.7	6-8	6-8	7.5	6.8	6-8
19 days.....		13-15	13-15		9-12	9-12		13-15	13-15
2½ months.....		35-45	Dead.		18-28	18-28		15-18	15-18

TABLE 3.—Results of inoculating *Ricinus* plants with *Bacterium tumefaciens* (hop strain) grown four days in 1:10 to 1:10,000,000,000 dilutions of filtrates of *Bacterium tumefaciens* (hop strain) of normal *Ricinus* plants, and of necrosed *Ricinus* tumors, respectively—Continued

Dilution and period between inoculation and examination.	Filtrate of beef-bouillon culture of hop strain (pH 6.7)			Filtrate of normal <i>Ricinus</i> plants (pH 5.4)			Filtrate of necrosed <i>Ricinus</i> tumors (pH 6.8)		
	Initial pH	Diameter of tumors on—		Initial pH	Diameter of tumors on—		Initial pH	Diameter of tumors on—	
		Plant No. 1	Plant No. 2		Plant No. 1	Plant No. 2		Plant No. 1	Plant No. 2
1 to 100:		<i>Mm.</i>	<i>Mm.</i>		<i>Mm.</i>	<i>Mm.</i>		<i>Mm.</i>	<i>Mm.</i>
10 days	7.7	9-12	9-12	7.7	6-8	6-8	7.5	6-8	6-8
19 days		13-15	13-15		9-12	9-12		13-15	13-15
2½ months		30-35	Dead.		35-45	18-28		15-18	15-18
1 to 1,000:									
10 days	7.5	9-12	9-12	7.2	6-8	6-8	8.1	6-8	6-8
19 days		13-15	13-15		6-8	6-8		9-12	9-12
2½ months		28-30	15-18		35-45	35-45		15-18	Dead.
1 to 10,000:									
10 days	7.5	9-12	9-12	7.6	6-8	6-8	8.1	6-8	6-8
19 days		9-12	9-12		9-12	9-12		13-15	9-12
2½ months		35-45	15-18		35-45	18-28		18-28	18-28
1 to 100,000:									
10 days	7.5	9-12	9-12	7.8	6-8	6-8	8.2	6-8	6-8
19 days		13-15	9-12		13-15	9-12		9-12	6-8
2½ months		35-45	30-35		35-45	18-28		18-28	15-18
1 to 1,000,000:									
10 days	7.5	9-12	9-12	7.7	6-8	6-8	8.2	4-5	4-5
19 days		13-15	13-15		13-15	9-12		9-12	9-12
2½ months		30-35	30-35		18-28	18-28		18-28	18-28
1 to 10,000,000:									
10 days	7.5	9-12	9-12	7.7	6-8	6-8	7.7	4-5	4-5
19 days		13-15	13-15		9-12	9-12		9-12	9-12
2½ months		35-45	35-45		18-28	18-28		15-18	15-18
1 to 100,000,000:									
10 days	7.5	9-12	9-12	7.6	6-8	6-8	8.0	6-8	6-8
19 days		9-12	9-12		9-12	9-12		9-12	9-12
2½ months		35-45	30-35		35-45	35-45		18-28	15-18
1 to 1,000,000,000:									
10 days	7.5	9-12	9-12	7.7	6-8	6-8	7.4	6-8	6-8
19 days		9-12	9-12		13-15	13-15		9-12	6-8
2½ months		18-28	28-30		18-28	Dead.		18-28	9-12
1 to 10,000,000,000:									
10 days	7.5	9-12	9-12	7.7	6-8	4-5	7.7	6-8	6-8
19 days		13-15	9-12		13-15	9-12		9-12	9-12
2½ months		15-18	15-18		18-28	18-28		18-28	15-18
Control:									
10 days	7.3	6-8	6-8	7.3	6-8	6-8	7.3	6-8	6-8
19 days		9-12	9-12		9-12	9-12		9-12	9-12
2½ months		18-28	18-28		18-28	18-28		18-28	18-28

* Control culture 4 days old. The same control plant was used for each dilution series.

This time, as the filtrate was sterile, in 24 hours the growth in the filtrate tubes was equal to that in the controls but not greater. It duplicated that in the third-passage tests made when the filtrate was used immediately after the tumors were crushed. No bacteriophage plaques appeared on the plates. The direction of the pH change of the filtrate of this particular *Ricinus* tumor was to the more alkaline side, which is contrary to that usually found in the younger tumors.

FILTRATES OF OLD DRY RICINUS TUMORS

It was thought that an active bacteriophage might have prevented necrosis in the old *Ricinus* tumors, for even when 6 to 7 months old they were still sound, although more or less dry. Fifty

tumors were ground up, and the juice was extracted and filtered. The hop strain was exposed to 2, 10, and 30 drops of this filtrate, and still other tests were made with the dry tumors. Some of the mashings were left in extract and infusion-beef bouillon for 5 and 24 hours, respectively. Each solution was filtered, and the hop strain was again exposed to 2, 10, and 30 drops of the different filtrates.

None of the filtrates of the dry tumor showed inhibition, and the bacteriophage plaques on the plates accompanying each test were very few and small or there were none at all. In each test there was refiltering with accompanying exposure of the hop strain, but still no inhibition or acceleration of growth as compared to that of the controls was noted.

FILTRATES OF SUGAR-BEET TUMORS

Tumors on the sugar beet were produced with the hop strain of *Bacterium tumefaciens*. They were crushed, the juice was filtered and 2, 10, and 30 drops of the filtrate were added to fresh beef-bouillon transfers of *Bact. tumefaciens*. Growth in the 2 and 10 drop cultures in 24 hours was heavier than in the control, while that in the 30-drop culture was retarded. In order to speed up the potency of the inhibiting factor, one of the 30-drop cultures was filtered and 2, 10, and 30 drops of this were added to a fresh beef-bouillon culture of *Bact. tumefaciens*. These filterings were carried through five passages, and while plaques appeared on the agar plates and slants, indicating the presence of the bacteriophage, the inhibition in the cultures lasted only 2 to 3 days. Hardened agar plates streaked with cultures made from several of the refiltered and inoculated cultures also showed the bacteriophage plaques.

Some of the plaques were transferred to fresh beef-bouillon cultures of *Bacterium tumefaciens*, but no difference was noted between the plaque-containing culture and the controls. The bacteriophage did not seem to be potent enough to cause lysis or even to retard cultures.

Ricinus plants were inoculated with the 10-drop and with one of the two 30-drop cultures of the original filtrate and organism. These inoculations produced tumors somewhat more rapidly than the controls and for about three weeks they were larger. In one month, however, the tumors in the controls equaled them in size.

A filtrate of other sugar-beet tumors (hop strain), oxidized for 12 days, in which the pH had changed from 6.2 to 5.0, was also used with *Bacterium tumefaciens*. Ten drops of the filtrate added to a transfer from a 24-hour culture of the hop strain produced a culture that had an unusual appearance. In the 10 days that it was under observation no pellicle formed, but there was a heavy precipitate in the bottom of the tube. The precipitate was not viscid as such precipitates usually are, and there was a heavy suspension of growth throughout the culture. Inoculations into Ricinus plants were made with the culture when it was 10 days old. The inhibiting factor evidently was present, for none of the Ricinus plants inoculated showed any outgrowths until after 5 weeks; then tumors appeared and developed very slowly. In 2 months they were only 1 cm. in diameter, while the control tumors were 4 cm. in diameter.

SEWAGE FILTRATE

A sewage filtrate was also used in the 2, 10, and 30 drop method with the hop strain. There were many refilterings, and the filtrate was added each time to a freshly seeded beef-bouillon culture. Retardation of growth did not occur until the third passage, and it did not last 48 hours. Filterings were continued with the use of one of the bacteriophage plaques from the third-passage plates in culture with the organism. The passages which followed produced more of the bacteriophage plaques on plates and slants, but retardation in the tube cultures was about the same. In the fifth passage of the plaque filterings growth was more rapid in the tube cultures than in the controls, and the plaques on the plates practically disappeared.

To ascertain whether this acceleration of growth in the tubes could be demonstrated in tumors on plants, *Ricinus* plants were inoculated with the 10 and 30 drop cultures. At the same time other *Ricinus* plants of the same age were inoculated with control cultures, and still others with 10 and 30 drops of sugar-beet tumor filtrate grown with the hop strain. In a week the sewage-filtrate hop inoculations showed well-defined outgrowths 4 mm. in diameter, the beet-filtrate hop inoculations showed good swellings though smaller, while the control-culture inoculations showed mere surface swellings. These differences continued for nearly three weeks, but in four weeks the control and the beet-filtrate tumors had nearly reached the size of the sewage-filtrate tumors.

EFFECTS ON THE DAISY STRAIN OF REPEATED FILTERINGS WITH FILTRATES OF SUGAR-BEET TUMORS

The attempt to isolate an inhibitory substance from filtrates of sugar-beet tumor was continued with these filtrates in company with the daisy strain of *Bacterium tumefaciens*. A feature of this strain is that it does not grow so rapidly on beef agar and in beef bouillon as does the hop strain.

In the first two passages of the sugar-beet tumor filtrate and the daisy strain there was greater growth than in the controls, and no bacteriophage plaques appeared on the plates. Slight inhibition began in the third passage.

Marked inhibition of growth in the 2, 10, 30, and 60 drop filtrate tubes inoculated with the daisy strain occurred, and bacteriophage plaques appeared on the plates of the fourth passage. A plaque from one of these plates was transferred to a slightly clouded beef-bouillon culture of daisy strain, incubated, filtered, and further passages made with the daisy strain, sometimes two and three and at other times only one in eight hours. This was continued through 24 passages. The activity of this filtrate embracing the bacteriophage plaque was marked beginning with the second passage, for there was inhibition of growth in the tube cultures, and plaques appeared on the plates from the second through the eighteenth passage. At this stage an older agar suspension was used instead of the usual 18 to 24 hour culture. With this older culture the activity of the bacteriophage seemingly was submerged, for no plaques appeared on the plates from the nineteenth to the twenty-fourth passage, when the experiment was discontinued. Neither was there a trace of inhibition of growth

in the 2, 10, 30, and 60 drop cultures of the twentieth to the twenty-fourth passage, and only slight inhibition in the nineteenth.

Partial lysis occurred in the tenth passage. Inhibition of growth continued up to the twelfth day in the 30-drop tube, then growth began, but there was only faint clouding with no formation of pellicle. Dead bacteria were in a precipitate at the bottom of the tube, and there was no motion in those alive in the suspended medium when examined in hanging drops.

Ricinus plants were inoculated with a portion of the tenth-passage inhibited culture, and other Ricinus plants were inoculated with a control culture of the same age. Tumors appeared on the control plants, but none on those inoculated with the inhibited culture. Examination of plants was made as late as two and one-half months after the inoculations.

The eleventh passage of the daisy strain with the sugar-beet tumor also showed inhibition in the 30-drop culture. Cowpeas were inoculated with this culture after growth took place, but no tumors resulted. The control inoculations gave small tumors.

Inhibition was not so marked in the twelfth to the eighteenth passages.

Some of the inhibited tenth-passage culture was filtered and treated in a series of new passages with the daisy strain. Much was expected of this filtrate, but no trace of inhibition was shown in two passages. This may have been due to the fact that an older culture of daisy was used for the initial passages, as an 18 to 24 hour culture was not available. It is quite essential to use a 24-hour or less beef-bouillon or agar culture suspension in beef bouillon.

EFFECTS OF FILTRATES OF ROTTED CARROTS ON THE HOP STRAIN OF BACTERIUM TUMEFACIENS AND OTHER ORGANISMS

When *Bacterium tumefaciens* (hop strain) was exposed to rotted-carrot filtrate and carried through a number of refilterings, the inhibition was longer than when it was carried through successive refilterings with tumor filtrates.

Sound carrots were inoculated with a pure culture of *Bacillus carotovorus* and kept in a moist chamber until a quantity of juice from the rotting roots had collected. This was filtered, and by means of poured plates it was found to be sterile. The filtrate was added to cultures of the following organisms to test their susceptibility to it: *Bacterium tumefaciens* (hop, peach, and daisy strains), *Bacillus carotovorus*, *B. mycoides*, *B. coli*, and *B. phytophthorus*. Loop transfers were made from a 24-hour beef-bouillon culture of all but *B. mycoides*, which had too heavy a growth in 24 hours, so a 5-hour culture of it was used. Transfers were made to pH 7.4 beef bouillon, and 2, 10, and 30 drops and 2 c. c. of the filtrate were added to the cultures. The hop strain of *Bact. tumefaciens* exposed to the 2 c. c. of filtrate was the only organism that showed inhibition. This inhibition lasted only three days. The effect of the filtrate continued, however, for the hop cultures did not produce pellicles, while the hop controls and the treated peach and daisy strains all had heavy pellicles.

The effect of 30 drops and 2 c. c. of the carrot filtrate on *Bacillus carotovorus*, the organism that rotted the carrot, was to produce a

slightly heavier growth than the control in 24 hours instead of inhibiting it.

Table 4 shows the effect of the carrot filtrate on the several organisms treated with it for 24 hours. In three days there was good growth in all the cultures that had shown slight growth except the hop strain. The hop strain then had slight growth in the 30-drop and 2 c. c. filtrate cultures as well as in the 2-drop and 10-drop ones.

TABLE 4.—*Effect of adding filtrate of carrots rotted with Bacillus carotovorus to cultures of seven different organisms*

Organism	Growth 24 hours after adding—				Growth in control culture after 24 hours
	2 drops	10 drops	30 drops	2 c. c.	
<i>Bacterium tumefaciens</i> :					
Hop strain	Slight	Slight	Questionable	None	Good.
Peach strain	Fair	Fair	Slight	Very slight	Do.
Daisy strain	Slight	do.	Good	Questionable	Do.
<i>B. carotovorus</i>	Fair	do.	Heavier than control.	Heavier than control.	Heavy.
<i>B. mycoides</i>	Heavy	Heavy	Fair	Fair	Do.
<i>B. coli</i>	do.	do.	do.	do.	Do.
<i>B. phytophthorus</i>	do.	do.	do.	do.	Do.

The experiment described above was repeated with the three strains of *Bacterium tumefaciens*, more of the rotted-carrot filtrate being used. Beef-bouillon tubes, pH 7.4, were seeded with a loop of a 24-hour culture of each organism, and because of the small quantity of the filtrate, only 2, 4, and 10 drops of it were added.

The daisy and peach strains showed such slight inhibition after the third passage that they were discontinued. The hop strain, on the other hand, produced marked inhibition at once, and in 48 hours only the merest trace of growth was discernible in the 10-drop tube and a faint clouding in the 2 and 4 drop tubes, while the controls had heavy growth with pellicles. In four days the clouding in the 2, 4, and 10 drop tubes had disappeared, but two days later it returned, and a definite and continued growth followed.

While it still showed inhibition, one of the cultures was used for refiltering and exposure again to pH 7.0 beef-bouillon cultures of the hop strain, and 2, 10, 15, 20, 25, and 30 drops of filtrate were added to the fresh hop cultures. There was inhibition of growth in all for four days. Although the inhibition seemed to be a case of lysis and there was no trace of growth in the first refiltering with exposure to the organism, 1 and 2 drops of one of the inhibited cultures were added to slightly clouded fresh cultures of the hop strain. If the bacteriophage was potent, it was thought that it would clear up the clouding in these tubes. This did not occur, for there was a heavier growth in them in 24 hours. Later the inhibited culture itself became clouded.

The inhibition was more marked in the third passage than in the first and second, and freshly clouded cultures of hop strain were cleared by 10-drop and 1 c. c. additions of one of the inhibited cultures. The clearing, however, lasted for only three days, when clouding began.

The inhibition of cultures occurred likewise in the sixth and seventh passages for a few days, and during this time clouded cultures were

treated with them to see whether lysis would occur. In the sixth passage this clearing did not take place, but further growth was stopped for 3 days. In the seventh-passage tests there was a clearing in the two cultures under observation which lasted 1 and 2 days, but typical *Bacterium tumefaciens* growth began on the third and fourth days. The seventh-passage inhibited cultures were used against more clouded hop-strain cultures, and the clearing, followed later by clouding, was repeated. No lysis occurred throughout the series.

As the seventh-passage inhibited cultures were used up in testing their ability to produce lysis in the clouded cultures, a third experiment was started with a fresh lot of rotted-carrot juice which had been filtered several times. The pH of the juice was 9.6 after filtering.

Along with this series of passages of rotted-carrot filtrate and the organism, filtered juice of healthy normal carrot exposed to the hop strain of *Bacterium tumefaciens* was tested also. The passages with the two filtrates were carried with the hop strain only, and 1-day-old beef-bouillon cultures were used for seeding pH 6.7 beef-bouillon tubes to which the filtrates were added.

In the third passage of the rotted-carrot filtrate there was inhibition for 13 days and in the fourth passage for 20 days. The increase in inhibition did not continue, for it dropped to 6 days in the fifth passage, increased to 7 days in the sixth, and fell back to 6 days in the seventh, when the passages were discontinued.

The inhibition in the normal-carrot filtrate exposed to the hop strain was four days at the third passage but dropped back to two days in the fourth and fifth passages.

BACTERIOPHAGE PLAQUES

The bacteriophage plaques on beef-agar slants and plates were studied more extensively in the third experiment with the rotted-carrot filtrate. The plaques appeared on the normal-carrot filtrate plates and slants (fig. 2, A) exposed to the hop strain as well as on slants and plates poured from the rotted-carrot filtrate (fig. 3, A; fig. 4, A, C, D, E, F) exposed to the hop strain, although they were not so numerous or so well defined. In addition to these, definite bacteriophage plaques occurred on the control plates (fig. 2, B; fig. 3, B; fig. 4, B), a thing that had not occurred before in the work. Table 5 gives some of the details of the third experiment with the rotted-carrot filtrate.

The control plates and slants showed plaques only when they were made from cultures weakened by frequent transfers and grown in a medium not too favorable. Figure 4, C, illustrates an agar-slant control culture which showed no plaques, while the agar slants of the rotted-carrot filtrate, third passage with *Bacterium tumefaciens*, seeded in the same way (fig. 4, E and F) showed many.

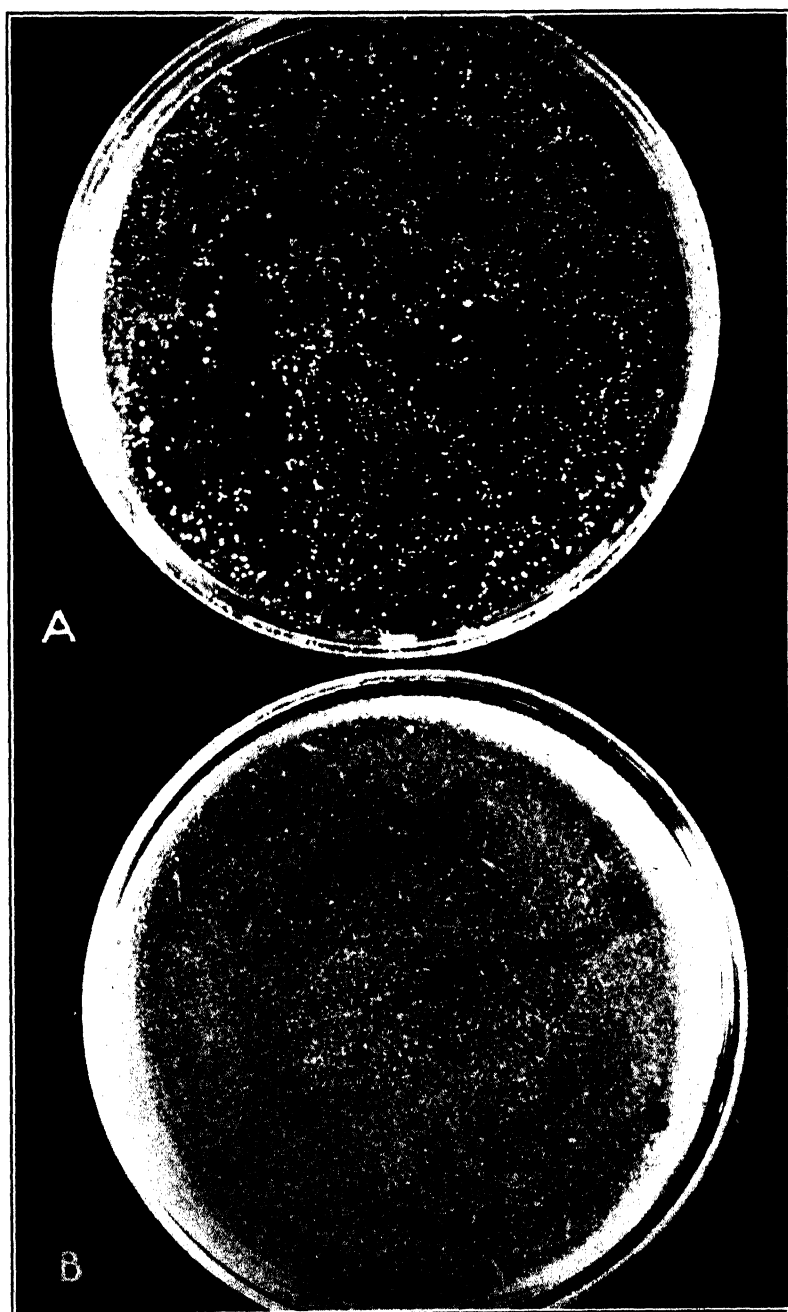


FIGURE 2.—Bacteriophage plaques on beef-agar plates: A, Sixth passage of *Bacterium tumefaciens* (hop strain) with filtrate of normal carrot; B, control, showing plaques less numerous but more striking than in A

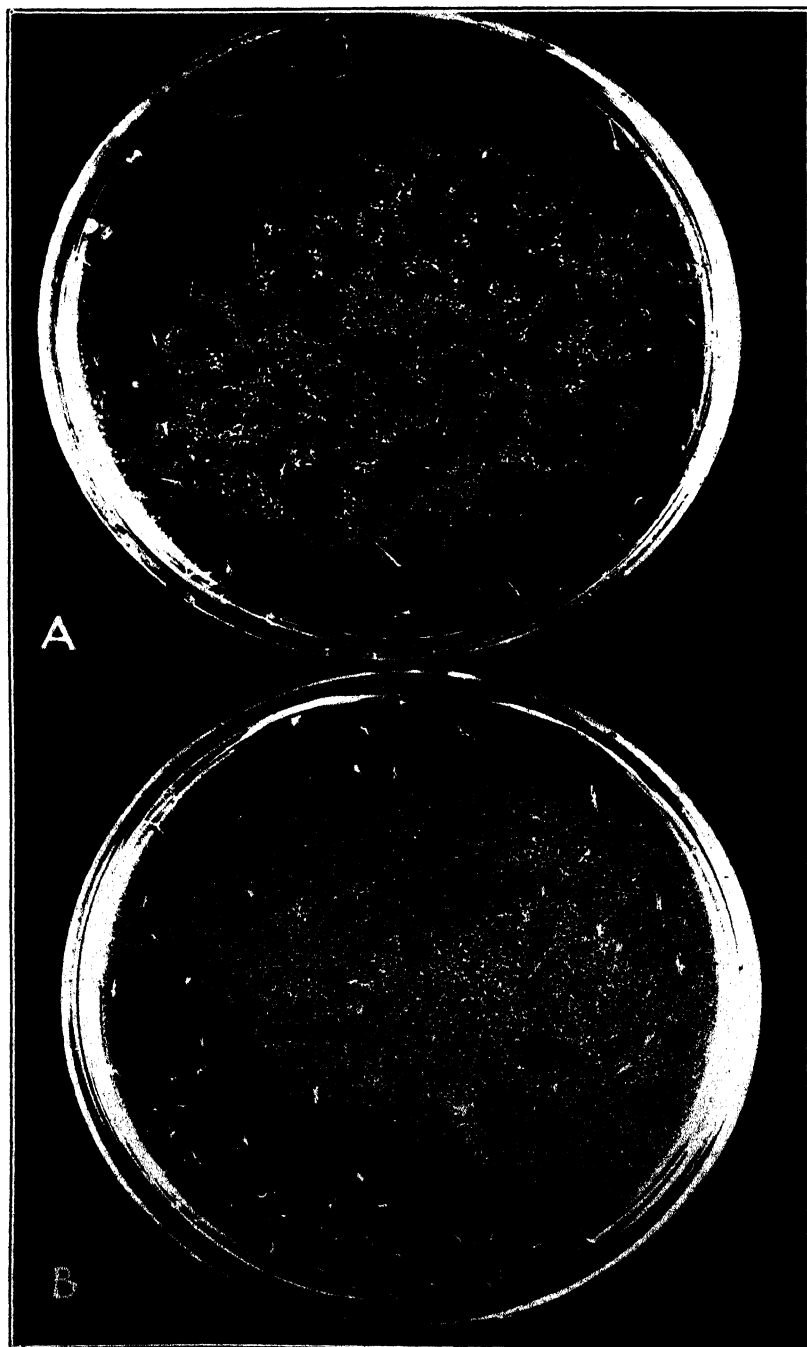


FIGURE 3.—Bacteriophage plaques on beef-agar plates with same amount of seeding: A, Fourth passage of *Bacterium tumefaciens* (hop strain) with filtrate of carrot rotted with *Bacillus carotovorus*; B, control plate of *Bact. tumefaciens* (hop strain), showing plaques also but not so numerous as in A.

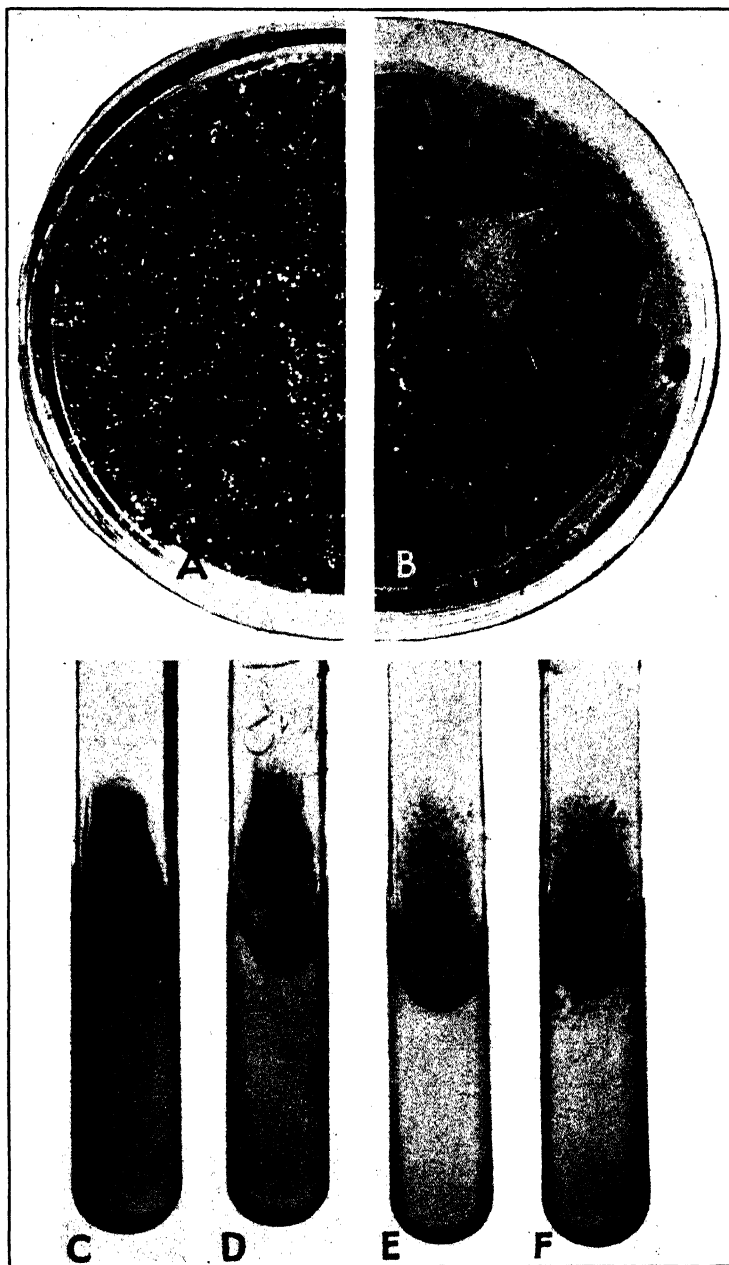


FIGURE 4.—Bacteriophage plaques of *Bacterium tumefaciens* (hop strain) on beef-agar plates and in tubes. A, Sixth passage of the organism with filtrate of rotted carrot. Note the presence of many plaques; B, Control. Note the presence of a few plaques; A and B received the same amount of seeding; C, Control culture seeded with two drops of a 24-hour beef-bouillon culture (plaques absent); D, Third passage of the organism with rotted-carrot filtrate swabbed on slant agar (tiny plaques present); E and F, Seeded with 2 drops of the third passage of the organism with the rotted-carrot filtrate (tiny plaques present)

TABLE 5.—*Effect of filtrates of rotted and normal carrots on Bacterium tumefaciens (hop strain) and the resulting bacteriophage plaques*

Passage	Filtrate of rotted carrot added to culture			Filtrate of normal carrot juice added to culture			Control culture in pH 6.7 beef bouillon	
	pH of filtrate before adding to culture, (pH 9.6 before passage with culture)	Inhibition (days)	Bacteriophage plaques	pH of filtrate before adding to culture (pH 6.8 before passage with culture)	Inhibition (days)	Bacteriophage plaques	Growth	Bacteriophage plaques
First.....	7.0	3	Few.....	7.1	1	Few.....	Good in 24 hours.	None.
Second.....	7.1	4	do.....	6.6	2	do.....	do.....	Do.
Third.....	6.8	13	More than with second passage.	6.7	4	do.....	do.....	Few.
Fourth.....	6.7	20	Abundant	6.7	2	Abundant	do.....	Moderate number; 25 on some plates.
Fifth.....	^a 7.8	6	do.....	6.0	2	None.....	do.....	Few and inconspicuous.
Sixth.....	6.2	7	do.....	6.2	^(b)	Many.....	^(c)	12 to 15 large ones on plates.
Seventh.....	6.7	6	Many, but not classic type.		^(b)		^(c)	Some, but not classic type.

^a Portion of filtrate stood open three days before reading was made.

^b Notes not taken for four days; good growth then.

^c Notes not taken for four days; heavy clouding then.

The plaques were tested in beef-bouillon cultures to see if they could produce lysis. Pieces of several of the fourth-passages plaques were cut out of the agar plates of the rotted and normal filtrates containing the organism and the control plates and were added to slightly clouded beef-bouillon cultures. The beef-bouillon did not clear, but there was no additional growth for two days, showing that the plaques had some inhibitive power. A definite clouding began on the third day or a little before that time in some cultures.

Sterile cover glasses were dropped on plaques on agar plates, removed, stained with carbol fuchsin, and studied under the microscope. The plaque picture was reproduced on the cover glass, for the tiny bacterial colonies outlining the plaques stained well. These colonies were made up mostly of swollen forms, very short rods appearing as coccus forms, and some normal-sized bacteria also, but the greater number of the normal ones were back from the margin of the plaque. A few bacteria in the center of the plaques appeared as tiny rods. These may have been drawn into the center by the removal of the cover glass from the top of agar plates, or they may have been there all the time. It is possible that they were bacteria inhibited but not destroyed by the bacteriophage. Among the swollen bacteria there were tiny particles which perhaps were the remains of swollen forms that had burst.

TESTS WITH INHIBITED CULTURES

Although the cultures of the fourth passage showed inhibition and it was thought that there was a condition of lysis, hanging drops from them were examined under the microscope 10 days after the cultures were made. Very few bacteria were located, but those found were typical rod-shaped forms of *Bacterium tumefaciens* without movement.

To study the phenomenon further, 10 drops of one of the inhibited cultures were added to slightly clouded beef-bouillon tubes. The clouding cleared somewhat in 1 day, but in 2 days definite *Bacterium tumefaciens* growth began and made slow but good development. Three days after these cultures were made the original fourth-passage cultures began to cloud, showing that there was no longer inhibition. The inhibition had lasted 20 days.

INOCULATIONS WITH INHIBITED CULTURES

The cultures inhibited 7, 13, and 20 days never developed typical *Bacterium tumefaciens* growth after growth began, but transfers from them to beef bouillon, pH 7.0, did. The clouding in the former was not heavy, no pellicle formed, and there was a precipitate of a less viscid consistency than usual at the bottom of the tube.

Ricinus plants were inoculated with the 7, 13, and 20 day inhibited cultures after growth took place in them, and also with control cultures of *Bacterium tumefaciens* made at the same time. These cultures of the sixth, third, and fourth passages, respectively, were the ones that demonstrated the bacteriophage plaques so well.

Power to infect was lost with the third passage, in which inhibition lasted 13 days, for no tumors formed (fig. 5 A), although the control cultures produced full-sized tumors (fig. 5, B).

Inoculations with the fourth passage, in which inhibition continued for 20 days, showed that some bacteria were still infectious, for tumors were produced. There was marked retardation in their development, however, for in 15 days 2 inoculations out of 5 did not show any developing outgrowth; the other three showed mere traces or swellings, while the 3 control culture tumors were 1.75 to 2 cm. in diameter. After nearly 3 months one inoculation was still negative; 2 of the tumors were only 2 cm. and 2 were 5 cm. in diameter (fig. 5, C), while the control tumors had reached their full size (5 cm.) in less than 2 months and before 3 months were necrosing. (Fig. 5, D.)

Inoculations with the sixth passage where there was inhibition for 7 days also showed a retardation of tumor formation and the presence of some infectious bacteria, as in the fourth passage. In one month two tumors of the sixth-passage inoculations were only 4 mm., while one was 12 mm., in diameter. The controls were 2 to 2.5 cm. in diameter. One inoculation was negative, and at the end of three months it continued negative. The smallest tumor was 12 mm., and the largest were 2 and 4 cm. (Fig. 5, E.) The control tumors at the same time were all 5 cm. in diameter. (Fig. 5, F.)

As the plants used for the 7, 13, and 20 day inhibited-culture inoculations were of the same age, inoculated about the same time and grown under the same conditions, the difference in the time of appearance of tumors produced by these cultures (or the nonappearance

of tumors) was striking, as was also the marked difference in the sizes of the tumors within a series, irrespective of the size of the controls. The bacteria inhibited for a time by the bacteriophage and later able

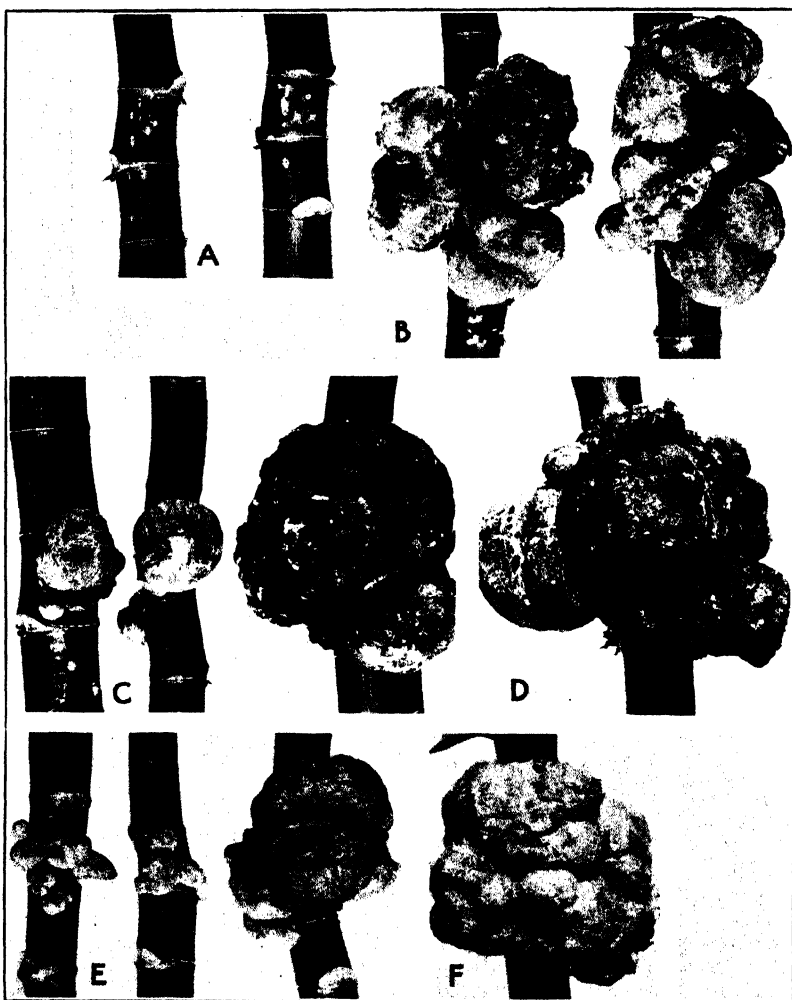


FIGURE 5.—Negative, retarded, and control infections produced by inoculating special cultures of *Bacterium tumefaciens* (hop strain) and control cultures of same organism into *Ricinus* plants. All reduced nearly one-half. A, Inoculation with third passage of *Bact. tumefaciens* and rotted-carrot filtrate. Photographed three months after inoculating; no infection. B, Control culture of *Bact. tumefaciens* inoculated into *Ricinus* plants. Photographed three months after inoculating. Regular-sized galls produced. Compare with A. C, Inoculations with fourth passage of the organism and rotted-carrot filtrate. Photographed more than three months after inoculating. Two tumors undersized and one nearly normal size. (One negative not shown.) D, Control culture inoculated into *Ricinus* and photographed at the same time as C. Tumor normal size. Compare with C. E, Inoculations with sixth passage of organism and rotted-carrot filtrate. Photographed three months after inoculating. Two tumors undersized and one nearly normal. (One negative not shown.) F, Control culture inoculated into *Ricinus* and photographed at the same time as E. Tumor normal size. Compare with E.

to overcome its action do not seem to retain their infectious ability to the same degree. A young normal culture of the hop strain of *Bacterium tumefaciens* inoculated into *Ricinus* gave 100 per cent

infection, the tumors varying but little in size when conditions were similar. The size of mature tumors produced by a normal culture of hop strain on *Ricinus* depended on the age of the plants at the time of inoculation, the time of year inoculated, and the rapidity with which the plants were grown. The size at maturity may range from 1.75 to 6 cm. Table 6 shows the results of the inoculations with the third, fourth, and sixth passage inhibited cultures after growth had taken place in the cultures.

TABLE 6.—*Results of inoculating Ricinus plants with equal-aged inhibited and control cultures of Bacterium tumefaciens*

Description of culture	Time after inoculation	Inoculation with inhibited cultures			Inoculation with control cultures		
		Plants inoculated	Tumors produced	Diameter of tumors	Plants inoculated	Tumors produced	Diameter of tumors
Third passage of <i>Bacterium tumefaciens</i> with rotted carrot filtrate in which growth did not begin until after 13 days of inhibition.		<i>Number</i>	<i>Number</i>	<i>(Mm.)</i>	<i>Number</i>	<i>Number</i>	<i>Mm.</i>
	1 month	4	0	—	3	3	20-25
	2 months	4	0	—	3	3	35-40
	3 months	4	0	—	3	3	45-50
	10 days	5	0	—	3	3	4-5
	15 days	5	3	2-3	3	3	17-20
Fourth passage in which growth was inhibited 20 days.	24 days	5	3	3, 4 and 12	3	3	40-45
	1 month, 17 days.	5	3	3, 20, and 25	3	3	50
	2 months, 23 days.	5	4	20 and 50	3	3	(^b)
	3 months, 15 days.	5	4	25 and 50	3	3	(^c)
	1 week	4	0	—	3	0	—
Sixth passage in which growth was inhibited 7 days.	1 month	4	3	4 and 12	3	3	20-25
	2 months, 4 days.	4	3	6, 12, and 25	3	3	45-50
	3 months	4	3	12, 20, and 40	3	3	50

^a Also one starting to swell.

^b Beginning to necrose.

^c Badly necrosed.

REPETITION OF D'HÉRELLE AND PEYRE'S EXPERIMENT IN WHICH THEY PRODUCED TUMORS BY A FILTERING FORM OF BACTERIUM TUMEFACIENS

In 1927 D'Hérelle and Peyre (4) published a report of some of their work with plant tumors produced by *Bacterium tumefaciens*. They believe that there are two kinds of colonies isolated from the tumor, one an ultrapure colony, nonparasitic, and another contaminated by the bacteriophage, which is the infectious colony. They believe also that the infection of the bacteria by the bacteriophage produces invisible forms of the bacteria, which they call protobacterial forms; and with the filtrate from plant tumors they have been able to obtain the return of the filtering protobacterial form to the normal bacterial form. Inasmuch as the microscopic examination of tumors does not show any bacteria, they suggest that the true parasite is the protobacterial form of the organism. These protobacterial forms they believe can act as virus filtrates and when inoculated into susceptible plants produce tumors. Their experiments with a filtrate from sugar-beet tumors induced by inoculating with *Bacterium tumefaciens* bore out this belief. Out of 20 sugar beets inoculated with the filtrate, 14 produced tumors within 70 days, and platings from 6 of the tumors gave typical cultures of *Bacterium tumefaciens*. According to D'Hé-

relle's hypothesis, the relation of the bacteria parasitized by the bacteriophage is a symbiotic one. This symbiosis has become stronger through continued passages through living hosts, and it is very difficult to break up, sometimes impossible with the methods now known.

The writers carried out the same experiment using *Ricinus* tumors for filtering. As soon as the filtrate was obtained, it was inoculated into 40 *Ricinus* plants with a hypodermic needle. The plants were kept under observation for several months, but no tumor appeared. The experiment was made three times and with three different lots of tumors. Each time other *Ricinus* plants of the same age were inoculated with a culture of *Bacterium tumefaciens* and held as controls. These control cultures always produced sizable tumors.

Poured plates of the three filtrates were made and studied for sterility. No colonies of *Bacterium tumefaciens* appeared. However, nine other colonies of several types were observed on the three sets, and, in the belief that these might be of value should there be a proto-bacterial form, they were transferred, cultured, and inoculated into *Ricinus* plants. No tumor arose from these vagrant colonies. In all probability they were air colonies that had no connection with the filtrates, and the filtrates themselves were free from *Bacterium tumefaciens*, as the hypodermic inoculations showed.

In order to test further the presence of an infectious filtering form of *Bacterium tumefaciens*, flasks of beef bouillon, pH 7.0, were inoculated with the hop strain, left to grow for 3 days, and then filtered. Sixteen *Ricinus* plants were inoculated hypodermically with some of the filtrate, and other *Ricinus* plants of the same age were inoculated with a 3-day-old control culture of the hop strain. Plates were poured from some of the filtrate to test its sterility. The plates were examined during a period of 11 days, but no colonies of *Bact. tumefaciens* appeared. The three colonies that came up on the plates were transferred, and inoculations into *Ricinus* were made with sub-cultures. The inoculations with both the sterile filtrate and the three suspicious colonies were kept under observation more than 2 months, but there was no trace of outgrowths. The control culture inoculations produced large tumors.

Part of the sterile beef-bouillon filtrate was held in the laboratory to give an opportunity for the filtering bacterial form to develop if such form occurs. There was a clouding in the filtrate in four days, but it was not a typical *Bacterium tumefaciens* growth. However, inoculations into *Ricinus* plants were made with the clouded filtrate because of the possibility that the growth might be a filtering infectious form of *Bact. tumefaciens* as D'Hérèlle and Peyre assert. These inoculations were watched for two and one-half months, but no trace of tumor growth occurred.

DISCUSSION

The conditions required for obtaining a culture of *Bacterium tumefaciens* that will produce more quickly growing and larger tumors than the controls can be brought about readily by adding a highly diluted tumor filtrate to a young culture of the organism and letting it grow two to four days before inoculating with it. There is some evidence

that filtrates of normal-plant juices also have the ability to produce more rapidly growing and larger tumors than the controls, although this evidence is based on experiments with normal *Ricinus* and normal carrot filtrates only.

A sewage filtrate combined with the organism produced faster growing and larger tumors than a filtrate of a sugar-beet tumor combined in the same way. The latter in turn produced more rapidly growing and larger tumors than the controls. A filtrate to which the organism was exposed as soon as possible after the tumor was crushed and filtered gave a culture which seemed to be nearly as effective in producing the rapid-growing and oversized tumors as a filtrate which had oxidized several days.

It may be that the addition of the fresh filtrate cements the union of the bacteria and the bacteriophage more closely and a higher degree of virulence is temporarily established.

No absolute lysis was produced in these experiments. It is quite possible, however, that the apple or rose strains of *Bacterium tumefaciens* might be cultured with some tumor filtrate and produce it. These two show evidence of being about the weakest strains of *Bact. tumefaciens*. This weakness, which manifests itself in producing tumors slowly when these strains are inoculated into susceptible plants, even into their native hosts, and the inability of these strains to produce tumors in some of the common plants easily grown in greenhouses, kept the writers from pursuing the development of lysis with the apple and rose strains through successive passages with a filtrate. The writers are assuming that the weakness of the strains is linked with the more facile separation of the bacteriophage from the organism. Very young cultures of the organism seemed to be necessary for the passages with the filtrates to produce inhibition. To obtain young cultures frequent transfers were required, which apparently had a tendency to bring about an instability of the close union that had become established between the bacteriophage and the organism and that allowed a release of the bacteriophage. In this temporary release the bacteriophage could affect the organism by its own power to cause inhibition. This release is manifested also by means of the bacteriophage plaques on agar slants and plates, and if a high potency has been reached, by marked inhibition. No doubt a condition could be brought about where there would be complete lysis. The culture that was inhibited for 13 days did not produce tumors when inoculated into *Ricinus* plants, although it was not used for inoculations until a few days after growth appeared. The control cultures of the same age produced tumors. An inhibited culture of 20 days produced tumors, retarded in appearing, but nevertheless present. The organisms resistant to the bacteriophage in the latter case were evidently of an infectious nature, while in the former they were not.

The bacteriophage plaques appearing on the plates poured from a pure culture of the hop strain of *Bacterium tumefaciens* as well as on those poured from filtrate *Bact. tumefaciens* cultures indicate that the lytic or inhibiting principle is carried along with the growth of the organisms irrespective of any active filtrate. The plaques from a pure culture were large and distinct but never so numerous as those on the filtrate-culture plates.

Sometimes there was a tumor-juice filtrate carried in passages with *Bacterium tumefaciens* which seemed not to activate the young culture, and if there was no inhibition of the organism in the early passages, it did not appear in a later passage. Whether the age of the tumor, the host of the tumor, or the rapid or slow rate at which the tumors had developed played a part in the presence and potency of the bacteriophage in the extracted juices was not fully determined. Frequently there was some evidence for thinking so. For instance, a sterile filtrate of old *Ricinus* tumors with necrosed areas gave no indication of inhibition when *Bact. tumefaciens* was exposed to it with three refilterings, nor at the beginning did it give evidence of producing acceleration of growth. The filtrates of very young tumors were not found satisfactory to work with. Whether this was because of the age or because the bacteriophage adhered to the more colloidal juice and did not pass through the filter is not known. It was found more difficult to filter the juice extracted from very young tumors. Those tumors not yet mature were found quite satisfactory and their filtrates could produce both inhibition and acceleration of growth when *Bact. tumefaciens* was exposed to them.

SUMMARY

The potentials of juices of normal and tumor tissue and *Bacterium tumefaciens* in culture revealed the following facts: (1) The pH of the freshly extracted juice of tumor tissue was always higher, that is, the reaction was more alkaline than that of the normal tissue; (2) the total acid content was greater in the tumor juice than in the normal juice; (3) the oxidation potential was always greater (more negative) in the freshly extracted tumor juice than in the normal juice—that is, the electrical charge carried by the gold electrodes in the fresh tumor juice was always negative (—) to that in the normal juice; (4) the electrical charges (accompanied always by a change in pH) on the gold electrodes were exactly reversed in the juices of the normal and tumor tissues upon further oxidation; (5) the change in pH produced by the growth of the organisms *Bact. tumefaciens* in beef-bouillon culture and *Bacillus carotovorus* inoculated into fresh carrots, progressed in the same direction (pH increase) as the change of pH of the normal juice upon further oxidation rather than in the direction (pH decrease) of the pH changes of the tumor juices; (6) the presence of a bacteriophage was established.

The presence or absence of plaques on poured plates appears to be correlated with the rise and fall of the pH of the plant filtrate or the pH of the culture of *Bacterium tumefaciens* treated with the filtrate. The pH value of the *Bact. tumefaciens* cultures which produced plaques when only 24 hours old was 6.7, and that of a normal *Bact. tumefaciens* culture 1 month old was 8.2. No plaques were present. A *Bact. tumefaciens* culture treated with a *Bacillus carotovorus* filtrate of pH 9.6 after 24 hours was pH 7.1 and after 4 days was pH 6.6, or slightly more acid than the fresh normal culture. This culture treated with *B. carotovorus* filtrate produced a greater number of plaques than *Bact. tumefaciens* cultures treated with other filtrates such as normal carrot, *Ricinus* tumor, or *Bact. tumefaciens*.

Accelerated growth of bacteria with increased pathogenicity seems to be associated with the phenomenon of bacteriophagy as well as does the retarded or inhibited growth of bacteria, causing either decreased or delayed pathogenicity or none at all.

The accelerated growth of the bacteria and increased size of tumors as compared with those of the controls were induced by the addition of high dilutions of tumor filtrates to young cultures used later for the inoculations. There is some evidence that filtrates of normal plant juices likewise have the ability to produce larger and more rapidly growing tumors than the controls. Inhibition of the bacteria from 4 to 20 days was obtained by successive passages of the tumor filtrate and light suspensions of the bacteria through Chamberland L3 filters. Tumors of small size or none at all were obtained through inoculations with these inhibited cultures after growth took place in them. Slight dilutions of the tumor filtrate grown with the culture inhibited the growth of bacteria and retarded tumor development. Tumors produced by these inhibited cultures occasionally reached the size of the control tumors, after a long slow growth. Complete lysis, the dissolving of all the bacteria in a culture, did not take place in the course of the work, which continued more than a year.

Bacteriophage plaques were obtained from the plant-tumor filtrates combined with *Bacterium tumefaciens*. The greatest number and largest ones, however, were obtained on beef-agar plates and slants from the rotted-carrot filtrate to which *Bact. tumefaciens* (hop strain) was exposed.

Typical bacteriophage plaques were obtained also on beef-agar plates and slants from a pure culture of *Bacterium tumefaciens*, and also in plates poured from a normal-carrot filtrate combined with *Bact. tumefaciens*. These plaques were not so numerous as those on the rotted-carrot filtrate plates.

D'Hérelle and Peyre assert that there exists a bacterial filtering form of *Bacterium tumefaciens* which is the infectious principle and which can be cultivated back to the nonfiltering form. Three repetitions of their experiment with sterile filtrates of *Bact. tumefaciens* tumors and also one with a sterile filtrate of beef-bouillon cultures of the organism were carried out, but the results were negative.

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NUTRITIVE VALUE OF PROTEINS IN CERTAIN KINDS OF SAUSAGE AND OTHER MEAT FOOD PRODUCTS¹

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INTRODUCTION

Sausage and other meat food products manufactured in whole or in part from chopped meat, meat by-products, and condiments occupy an important place in the dietary of the American people. The estimated total production of sausage and sausage casings in the United States for the year 1925 was approximately 1,366,849,000 pounds, exclusive of sausage manufactured by small retail butchers and provision dealers and by farmers. These figures, after correction for exports and imports, indicate a per capita consumption of 11.6 pounds per annum.²

Sausage and certain other meat food products are prepared chiefly from two groups of ingredients, viz, meat and meat by-products. The term "meat"³ denotes the flesh of cattle, swine, sheep, and goats. The meat used in the manufacture of sausage consists chiefly of trimmings from the carcasses of swine and cattle, but it may also include certain cuts and even entire carcasses of these animals. The term "meat by-product" denotes edible organs and parts, other than meat, obtained from the carcasses of cattle, swine, sheep, and goats. Characteristic condiments are used in the preparation of each kind of sausage. Cereal, usually corn flour, may be added to the extent of 3.5 per cent to sausage manufactured in federally inspected establishments provided the product is marked to indicate the presence of cereal. If products prepared in simulation of sausage contain substances foreign to sausage, or excessive quantities of cereal or moisture, they must be branded "Imitation."

In a general way, each kind of sausage or other meat food product is prepared from a certain group of ingredients, but the kinds and proportions used may vary considerably in different establishments. Some manufacturers prepare more than one grade of the same product, using a different formula for each grade. Naturally the best grade is made from the choicest materials.

There is much information in the literature concerning the chemical composition of sausage and other meat food products, but these data do not fully indicate the nutritive value of these products. Information concerning the vitamins in sausage and the biological value of sausage proteins is meager or lacking. The writers have already studied the nutritive value of the proteins in a number of kinds of

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² These figures have been calculated from data in reports of the Bureau of the Census and the Bureau of Foreign and Domestic Commerce, U. S. Department of Commerce, 1925.

³ UNITED STATES DEPARTMENT OF AGRICULTURE, OFFICE OF THE SECRETARY. MEATS AND THE PRINCIPAL MEAT PRODUCTS. U. S. Dept. Agr. Food Insp. Decision 205, 3 p. 1926.

meat and meat by-products⁴ used in the manufacture of sausage and have found considerable differences in the biological values of the proteins in certain of these products. It seemed likely, therefore, that material differences might be found in the nutritive values of the proteins in different kinds of sausage and other meat food products. The purpose of the experiments reported in this paper was to compare the nutritive values of the proteins in certain of the more common kinds of sausage and other meat food products.

DESCRIPTION OF MEAT FOOD PRODUCTS TESTED

The following meat food products were tested: Frankfurter-style sausage (two grades), Bologna-style sausage (two grades), pure pork sausage, fresh link sausage other than pure pork, Braunschweiger-style sausage, liver pudding or sausage, headcheese, scrapple, and meat loaf. For purposes of comparison, fresh pork ham and beef chuck were also tested. The above-named products were purchased direct from four meat-packing establishments operating under Federal inspection.

Frankfurter-style sausage of the best quality is prepared from meat, usually a mixture of beef and pork trimmings and certain beef cuts, and seasoning. The meat is ground very fine, mixed with salt and spices, and stuffed in animal or artificial casings. The sausage is then smoked, cooked for a short time in hot water, and finally chilled. Cheaper grades of Frankfurter-style sausage are made from a mixture of meat, meat by-products, and seasoning. Cereal is frequently added to these grades of sausage.

Bologna-style sausage is prepared from similar ingredients and in a like manner to Frankfurter-style sausage, except that the former is stuffed in larger casings or other containers and is cooked for a longer time. The cheaper grades of this product are made from much the same materials as those used for the same grades of Frankfurter-style sausage. Kosher Bologna-style sausage and kosher Frankfurter-style sausage contain no pork.

Pure pork sausage is prepared exclusively from pork and seasoning. The pork usually consists of lean-pork trimmings, but certain lean-pork cuts are used to a limited extent. The ground mixture is stuffed in animal casings or is packed in other containers.

Fresh link sausage, not pure pork, is made from beef and pork trimmings and seasoning, with or without the admixture of meat by-products. Cereal is frequently added.

Braunschweiger-style sausage is a high-grade liver sausage prepared from livers, pork trimmings, and seasoning. The product is thoroughly cooked and then stuffed in large hog casings.

Liver pudding or liver sausage is manufactured chiefly from meat by-products and may contain a moderate proportion of meat. Cereal is frequently added to this product. Liver pudding is thoroughly cooked in the process of manufacture.

⁴ HOAGLAND, R., and SNIDER, G. G. NUTRITIVE VALUE OF THE PROTEIN IN VEAL AND CALF SWEET-BREADS; IN BEEF CHEEK MEAT, LIPS, TONGUES, BRAINS, SPLEEN, AND TRIPE; AND IN HOG BRAINS AND TONGUES. *Jour. Agr. Research* 32: 679-688. 1926.

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Headcheese is usually prepared from a variety of meat by-products from swine and cattle, together with seasoning. This product may contain a moderate proportion of meat. Pig tongues are a frequent constituent of headcheese.

Scrapple is prepared chiefly from cereals, usually corn meal, buckwheat flour, or a mixture of the two with a variety of meat by-products, occasionally meat, and seasoning. The several ingredients are mixed with water, cooked to the desired consistence, and molded in pans. This product is sliced and fried before eating.

Meat loaf is usually prepared from a mixture of beef, pork, cereal, and seasoning. Occasionally veal is used. The finely ground mixture is molded in pans and thoroughly cooked. Loaves, other than true meat loaves, are prepared from a mixture of meat, meat by-products, and cereal.

METHODS

The procedure followed in these experiments was essentially the same as that previously used by the writers in a study of the nutritive value of the proteins in various animal tissues.⁵ The proteins in the several kinds of sausage and other meat food products were compared by feeding tests with young male albino⁶ rats. Each ration was fed for 60 days to 6 rats weighing approximately 40 gm. each and not exceeding 30 days in age at the beginning of the test. The rats fed each ration were selected from three litters. The animals were weighed twice weekly and an accurate record was kept of the feed consumed by each.

Protein was fed at the 10 per cent level. Sufficient dried meat food product was used in each ration to furnish the desired proportion of protein and the rations were made adequate in other respects for normal growth. In addition to the meat food product, each ration contained the following constituents: Ash mixture, 4 per cent; cod-liver oil, 2 per cent; concentrated yeast extract, 2 per cent; hydrogenated cottonseed oil, 8 per cent; and sufficient cassava starch to make 100 per cent. The starch was practically free from nitrogen, but the yeast extract contained approximately 8 per cent nitrogen.

The meat food products were first separated from their containers, ground fine, and dried in a current of warm air. The dried products were ground, thoroughly extracted with ether, and analyzed for nitrogen.

RESULTS OF EXPERIMENTS

In Table 1 are presented the results of the first 30 days' feeding, and in Table 2 are given the data for the entire 60-day test. Only the average data for each group of six rats are recorded.

Ten per cent of meat protein of high biological value is not quite sufficient for normal growth in rats under the conditions of these experiments. Hence, material differences in the nutritive values of the proteins of the various meat food products were readily detected. Gain in weight without reference to protein intake is, of course, not an adequate basis for comparison.

⁵ HOAGLAND, R., and SNIDER, G. C. THE VALUE OF BEEF PROTEINS AS A SUPPLEMENT TO THE PROTEINS IN CERTAIN VEGETABLE PRODUCTS. *Jour. Agr. Research* 34: 297-308. 1927.

TABLE 1.—*Nutritive value of protein in sausage and other meat food products, compared with that in beef and pork, when fed to young male albino rats for a period of 30 days at a protein level of 10 per cent*

Source of proteins *	Average age and weight of experimental animals			Total food intake		Intake per gram gain in weight		Gain in weight per gram consumed	
	Age at start	Weight at start	Gain in weight in 30 days	Entire ration	Protein	Entire ration	Protein	Entire ration	Protein
No. 1 grade Frankfurter-style sausage, establishment A...	29	40	43	180	18.0	4.19	0.42	0.24	2.39
Do.....	28	40	44	188	18.8	4.27	.43	.23	2.34
No. 1 grade Frankfurter-style sausage, establishment B.....	24	42	85	302	30.2	3.55	.36	.28	2.81
No. 2 grade Frankfurter-style sausage, establishment C.....	27	40	60	234	23.4	3.90	.39	.26	2.56
No. 1 grade Bologna-style sausage, establishment A.....	24	39	58	248	24.8	4.28	.43	.23	2.34
No. 1 grade Bologna-style sausage, establishment B.....	23	41	74	268	26.8	3.62	.36	.28	2.76
No. 2 grade Bologna-style sausage, establishment C.....	25	44	66	245	24.5	3.71	.37	.27	2.69
Liver sausage, establishment A.....	26	43	49	230	23.0	4.69	.47	.21	2.13
Liver sausage, establishment B.....	26	42	64	285	28.5	4.45	.45	.22	2.25
Braunschweiger-style sausage, establishment A.....	26	43	68	211	21.1	3.10	.31	.32	3.22
Braunschweiger-style sausage, establishment B.....	25	45	100	308	30.8	3.08	.31	.32	3.25
Pork sausage, establishment A.....	28	40	76	223	22.3	2.93	.29	.34	3.41
Pork sausage, establishment B.....	26	41	70	227	22.7	3.24	.32	.31	3.08
Fresh link sausage, establishment D.....	23	39	41	186	18.6	4.54	.45	.22	2.20
Beef chuck (cooked), establishment A.....	26	41	95	324	32.4	3.41	.34	.29	2.93
Beef chuck (uncooked), establishment A.....	27	41	101	329	32.9	3.26	.33	.31	3.07
Pork (fresh ham), establishment B.....	25	40	125	340	34.0	2.72	.27	.37	3.68
Do.....	26	41	102	307	30.7	3.01	.30	.33	3.32
Meat loaf, establishment A.....	25	41	62	199	19.9	3.21	.32	.31	3.12
Meat loaf, establishment B.....	25	39	107	308	30.8	2.88	.29	.35	3.47
Scrapple, establishment A.....	27	44	34	172	17.2	5.06	.51	.20	1.98
Scrapple, establishment B.....	27	42	74	258	25.8	3.49	.35	.29	2.87
Headcheese, establishment A.....	27	41	26	165	16.5	6.35	.63	.16	1.58
Headcheese, establishment B.....	26	41	34	175	17.5	5.15	.51	.19	1.94

* Each lot of sausage or other meat food product was fed to six rats; results given are averages.

From the data presented in Table 1, the meat food products may be divided into three groups based on the apparent nutritive values of the proteins in those products. In the first group are included products containing proteins which showed the highest nutritive values, viz, pork sausage, Braunschweiger-style sausage, and meat loaf. In the second group are included products containing proteins which exhibited intermediate values, viz, Frankfurter-style sausage and Bologna-style sausage. Scrapple from establishment E also belongs in this group. In the third group are included those products containing proteins which seemed to be of lower nutritive value than those of the first two groups, viz, liver sausage, headcheese, and fresh link sausage. Scrapple from establishment A is included in this group.

For the first group, the gain in weight for each gram of protein consumed ranges from 3.08 gm. for pork sausage, establishment B, to 3.47 gm. for meat loaf, establishment B. The values obtained for the products in this group compare favorably with those recorded for beef chuck and fresh ham, viz, 2.93 to 3.68 gm.

For the second group the gain in weight per gram of protein consumed ranges from 2.34 gm. for Frankfurter-style sausage, establishment A, to 2.87 gm. for scrapple, establishment E.

For the third group the gain in weight for each gram of protein consumed ranges from 1.58 gm. for headcheese, establishment A, to 2.25 gm. for liver sausage, establishment B.

TABLE 2.—*Nutritive value of protein in sausage and other meat food products, compared with that in beef and pork, when fed to young male albino rats for a period of 60 days at a protein level of 10 per cent*

Source of proteins ^a	Average age and weight of experimental animals			Total food intake		Intake per gram gain in weight		Gain in weight per gram consumed	
	Age at start	Weight at start	Gain in weight in 60 days	Entire ration	Protein	Entire ration	Protein	Entire ration	Protein
	Days	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams
No. 1 grade Frankfurter-style sausage, establishment A.....	29	40	96	468	46.8	4.88	0.49	0.21	2.05
Do.....	28	40	95	470	47.0	4.95	.49	.20	2.02
No. 1 grade Frankfurter-style sausage, establishment B.....	24	42	171	697	69.7	4.08	.41	.25	2.45
No. 2 grade Frankfurter-style sausage, establishment C.....	27	40	114	502	50.2	4.40	.44	.23	2.27
No. 1 grade Bologna-style sausage, establishment A.....	24	39	117	596	59.6	5.09	.51	.20	1.96
No. 1 grade Bologna-style sausage, establishment B.....	23	41	152	616	61.6	4.05	.41	.25	2.47
No. 2 grade Bologna-style sausage, establishment C.....	25	44	130	565	56.5	4.35	.43	.23	2.30
Liver sausage, establishment A.....	26	43	87	512	51.2	5.89	.59	.17	1.70
Liver sausage, establishment B.....	26	42	122	655	65.5	5.37	.54	.19	1.86
Braunschweiger-style sausage, establishment A.....	26	43	141	578	57.8	4.10	.41	.24	2.44
Braunschweiger-style sausage, establishment B.....	25	45	175	725	72.5	4.14	.41	.24	2.41
Pork sausage, establishment A.....	28	40	152	556	55.6	3.66	.37	.27	2.73
Pork sausage, establishment B.....	26	41	156	588	58.8	3.77	.38	.27	2.65
Fresh link sausage, establishment D.....	23	39	90	462	46.2	5.13	.51	.19	1.95
Beef chuck (cooked), establishment A.....	26	41	160	689	68.9	4.31	.43	.23	2.32
Beef chuck (uncooked), establishment A.....	27	41	166	693	69.3	4.17	.42	.24	2.40
Pork (fresh ham), establishment B.....	25	40	201	731	73.1	3.64	.36	.27	2.75
Do.....	26	41	193	727	72.7	3.77	.38	.27	2.65
Meat loaf, establishment A.....	25	41	135	523	52.3	3.87	.39	.26	2.58
Meat loaf, establishment B.....	25	39	186	716	71.6	3.85	.38	.26	2.60
Scrapple, establishment A.....	27	44	77	435	43.5	5.65	.56	.18	1.77
Scrapple, establishment E.....	27	42	137	543	54.3	3.96	.40	.25	2.52
Headcheese, establishment A.....	27	41	60	398	39.8	6.63	.66	.15	1.51
Headcheese, establishment B.....	26	41	67	402	40.2	6.0	.60	.17	1.67

^a Each lot of sausage or other meat food product was fed to 6 rats; results given are averages.

If the meat food products are again classified according to the relative nutritive values of their proteins, based on the data for the 60-day experiments, the arrangement is slightly different, as follows: Group 1 includes pork sausage, Braunschweiger-style sausage, meat loaf, Frankfurter-style sausage, and Bologna-style sausage from establishment B, and scrapple from establishment E. Group 2 includes Frankfurter-style and Bologna-style sausage from establishments A and C. Group 3 embraces headcheese, fresh link sausage, and liver sausage. Scrapple from establishment A is included in this group.

For the first group the gain in weight for each gram of protein consumed during the 60-day test ranges from 2.41 gm. for Braunschweiger-style sausage, establishment B, to 2.73 gm. for pork sausage, establishment A. The range of values for this group compares favorably with that for beef chuck and pork hams, viz, 2.32 and 2.75 gm.

For the second group the gains for the 60-day period range from 1.96 gm. for Bologna-style sausage, establishment A, to 2.30 gm. for the same kind of sausage from establishment C.

For the third group, the gain in weight for each gram of protein consumed ranges from 1.51 gm. for headcheese, establishment A, to 1.95 gm. for fresh link sausage, establishment D.

DISCUSSION OF RESULTS

The results of the experiments reported in this paper indicate material differences in the apparent nutritive values of the proteins in the several meat food products examined. Based on these results, the products have been classified in three groups, but this arrangement applies only to the samples tested and does not have general application.

Attention is called to the fact that, among products from the same establishment, the proteins in Frankfurter-style sausage and in Bologna-style sausage had practically the same apparent nutritive value. These results were to have been expected because each establishment ordinarily makes the two kinds of sausage from essentially the same mixture of meats. It may be noted, however, that there are material differences in the nutritive values of the proteins in different lots of these two sausages purchased from different establishments.

The values obtained for the proteins in cooked and uncooked beef chuck, both samples being from adjacent parts of the same quarter of beef, agreed very closely for both the 30 and 60 day tests. The results obtained for two lots of fresh ham purchased at different times from the same establishment did not agree very well for the 30-day test but were very similar for the 60-day test. On the whole, the results indicate that the procedure followed in these experiments has given similar results with like products.

Considerable difference was found between the nutritive values of the proteins in the two lots of scrapple. During the 60-day test the rats getting scrapple from establishment A gained only 1.77 gm. in weight for each gram of protein consumed, whereas those fed scrapple from establishment E gained 2.52 gm. This difference is probably due to the use of a larger proportion of lean meat in the manufacture of scrapple at establishment E. It is noteworthy that, for the 60-day test, the protein in scrapple from establishment E had as high nutritive value as that in beef chuck. This fact is doubtless due to the supplemental relationship between the proteins of meat and those of corn meal, since both of these products were used in the manufacture of this lot of scrapple. The writers have previously reported concerning the value of beef protein as a supplement to that in corn meal.⁶

⁶ HOAGLAND, R., and SNIDER, G. G. Op. cit. (See footnote 5.)

Attention has been called to the fact that, although each kind of sausage or other meat food product is usually made from a certain group of ingredients, there is nevertheless considerable variation in the practice at different meat-packing establishments. Not only may there be variations in the kinds and proportions of the different meats and meat by-products used, but there may also be differences in the quality of the ingredients as well. It is thus obvious that considerable variation may occur in the nutritive values of the proteins in the same type of sausage manufactured at different establishments. Hence the results of the experiments reported in this paper can not be taken as a basis for generalizations concerning the nutritive values of the proteins in the various products examined.

SUMMARY

The nutritive values of the proteins in certain kinds of sausage and other meat food products have been compared with those of beef chuck and fresh pork ham by feeding experiments with young albino rats. The results of these tests show that the proteins in certain products were of considerably higher nutritive value than the proteins in other products. The nutritive values of the proteins in samples of the same product manufactured by different establishments also varied.

Based on similarity in the nutritive values of their proteins, the meat products examined may be arranged in three groups. Group 1 includes those products containing proteins of the highest nutritive value, viz, all samples of pork sausage, Braunschweiger-style sausage, and meat loaf. The proteins in these products had practically the same nutritive value as those in beef chuck and fresh pork ham. Group 2 comprises products with proteins of lower nutritive value than those in Group 1, as follows: All samples of Frankfurter-style and Bologna-style sausage and one lot of scrapple from establishment E. Group 3 includes products with proteins of lower nutritive value than those in Group 2, viz, all samples of headcheese, liver sausage, and fresh link sausage, and one lot of scrapple from establishment A.

ARTIFICIAL SOILING OF COTTON FABRICS PREPARATORY TO LAUNDERING STUDIES¹

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INTRODUCTION

Any scientific investigation dealing with laundering requires cloth soiled in a definite manner with known constituents. The study here reported was undertaken in order to develop a soiling mixture for use in experimental work on laundering being done in this laboratory. In this connection it was also necessary to study methods of applying the mixture to the fabric and of determining the amount of soil on the cloth after soiling and after the various laundering processes. Naturally soiled fabrics are not satisfactory because the nature and the quantity of the soil are not known, the soil is not distributed in a uniform screen, and the depth of the soil is not great enough to permit a gradation of grayness with variations in the washing process.

DISCUSSION OF FORMULAS AND METHODS USED BY OTHER INVESTIGATORS

Excluding stains, which have to be treated aside from the regular laundry process, there are four kinds of dirt commonly found on soiled clothing and household textiles: Albuminous material, such as eggs, blood, and body excretions; finely divided matter, such as soot and dust; saponifiable oils, including those of both animal and vegetable origin; and nonsaponifiable oils, such as mineral oils. Artificial soiling mixtures used by other experimental workers have contained substances that belong to one or more of these classes of compounds. Table 1 incorporates such formulas as have been found in the literature and obtained by private communication.

The choice of the component of the soiling mixture that gives the gray color to the test cloth has been the most troublesome problem. It must be chemically inert and insoluble in the detergents used, for any whitening should be an indication of the removal of soil by the washing process. Lampblack, carbon black, linden charcoal, benzol soot, and graphite have been used by the various investigators. (Table 1.) Elledge and Isherwood (12),² consider lampblack a substance comparable to soil in clothes since it consists of finely divided particles of carbon with various hydrocarbons condensed on them. However, it is rather unreliable in that each new supply may exhibit different characteristics. The American Oil Chemists' Society tried a suspension of lampblack in carbon tetrachloride as noted in formula

¹ Received for publication Mar. 23, 1929; issued October, 1929.

² Reference is made by number, (italic), to "Literature cited," p. 549.

5A (Table 1) but found it unsatisfactory. Formula 5C is now being used.

The use of carbon black, linden charcoal, and benzol soot may have been attempts on the part of the different investigators to employ a substance more constant in composition than lampblack. By microscopical examination, Butcher (?) finds that particles of lampblack (0.3–0.4 micron) are noticeably larger than those of carbon black (0.1–0.15). There is also a distinct difference between the two in that the latter mixes far more readily with water. Carbon black would therefore be favored above lampblack because its particles are smaller and it is more easily dispersed in water. A very useful form was found in news ink, which is a mixture of 10 per cent carbon black and 90 per cent mineral oil.

Graphite has been used in preference to lampblack in formulas 3, 12 and 16 (Table 1), and Chapin (8, *p.* 461) states that it has the advantage over any other form of carbon for detergency tests since it is free from porosity, and equilibrium in adsorption is reached in far briefer time. The British Launderers' Research Association, according to Parker (28), suggested the use of commercial preparations that eliminate the difficulty of wetting lampblack or finely ground dry carbon. One of these is available on the market as a suspension in water known as Aquadag, and another as a suspension in oil called Oildag. According to the manufacturer, they contain approximately 20 per cent and 12.5 per cent, respectively, of deflocculated graphite. These figures are higher than those reported by Parker (28). The extremely fine subdivision of the deflocculated graphite, which is in colloidal form, also makes it valuable for use in a soiling mixture. Aquadag is not so useful as Oildag for this purpose because of the greater difficulty in removing it from the fabric. Furthermore, its application is time-consuming, since a second dip in an organic solution of the oils is necessary after it has dried on the cloth.

Heermann (14) suggested the use of indigo paste instead of any form of dry carbon because this paste is always available in the same chemical composition and in the same mechanical form. Hirose (18) also reports the use of indigo as a component of his soiling mixture. However, if photometric readings are made to determine the amount of dirt removed by the washing process, blues are not suitable for comparison with a white standard. A black dye might be substituted for the indigo paste, but the question arises as to the difference in the power of hot and cold water to break down the mechanical attachment of the dye and the fiber. The same objection as to color can be raised against the reddish-brown burnt umber which was used in formula 9. (Table 1.)

There has also been a difference of opinion among investigators as to the most desirable oil or fat to include in the soiling mixture. Lindner (23, 24) believes that detergents that are good oil washers remove plant oils, animal fats, petroleum, resin, and, in fact, any organic substances of liquid or semiliquid nature just as well as mineral oil. He differentiates only between their "soot washing power" and their "oil washing power." In addition, present theories (Hillyer, 15, 16, 17; Spring, 33), are that soap cleanses by virtue of the undecomposed soap through emulsification, absorption, and the lowering of the interfacial surface tension at the oil-water interface, not because

of the saponifying power (McBain, 25, 26, 27) of the small quantity of alkali set free by the hydrolysis of even a neutral soap in aqueous solution. It is therefore probably unnecessary to include more than one oil to demonstrate the oil-removing power of the detergent employed, but an artificial soiling mixture which will simulate natural soil should perhaps contain a saponifiable animal oil, a saponifiable vegetable oil, and an unsaponifiable oil.

In choosing a representative of saponifiable oils, the standard groups—drying, nondrying, and semidrying—might be utilized, but this apparently has not been done in the formulas cited in Table 1. Of the animal fats used, tallow seems to be as satisfactory as any and is readily obtainable. High-quality olive oil is the least oxidizable of all vegetable oils, and is therefore an ideal representative of this group for a soiling mixture. Lanolin, though used in two of these formulas, is an animal wax rather than a fat or an oil. It would be useful as a component of a soiling mixture for wool-scouring investigations rather than for laundering experiments, since it is rarely a part of natural soil.

The only factor governing the selection of different mineral oils when they are included in the soiling mixture seems to be the color. A colored oil may introduce a yellow tone into the gray of the carbon and so make photometric readings more difficult. Refined oils are preferable since they may be better standardized.

The components of the dirt mixture should be dispersed in an oil-dissolving organic liquid, nonflammable, and reasonably volatile, with its boiling point somewhat below the boiling point of water but far above room temperature. Such a solvent permits the application of the inert and oily substances in one process and dries rapidly enough to avoid streaking of the soiled samples. Carbon tetrachloride is the only desirable one reported.

A study of Table 1 reveals a range of 0.04 to 0.2 gm. of carbon per 100 c. c. of medium, furnishing, doubtless, a great variety of soiled test cloths. However, the depth of soil obtained is due not only to the differences in the amount of carbon but also to the kind of carbon and the technic of soiling. If the 25 per cent brightness, corresponding to shade S48 of the Munsell gray scale, as earlier suggested by the committee of the American Oil Chemists' Society (20) is accepted as satisfactory, the amount of carbon and the length of time of soiling can be determined by trial.

The quantity of saponifiable and unsaponifiable oils included in the soiling mixture seems to have been an arbitrary matter with each investigator. In six of the formulas cited the total oily constituents range from 0.2 to 5.0 gm. per 100 c. c. of medium. The maximums are exhibited by the formulas of Brauer (5), Chapin (10), Lindner (23, 24), and Shorter (31), who report the impregnation of the fabric with oil undiluted by any solvent. The important feature seems to be simply to have enough oil present to attach the inert matter to the fabric and thus to simulate natural soil. Equal quantities of saponifiable and nonsaponifiable oils would seem a fair test, the former being composed of equal weights of animal and vegetable oily matter.

A satisfactory method of applying soil to a test cloth deposits it in a uniform manner over the entire surface and permits reproduction in kind and depth of soil when another sample of the same cloth is

subjected to the treatment. The methods employed by different investigators comprise (1) agitation and shaking of the test cloth in the dirt mixture; (2) spreading the soil over the surface of the cloth; (3) passing the cloth through the soiling mixture; (4) printing in stripes; and (5) soaking the test cloth in the dirt mixture. Test cloths of a great variety of kinds and dimensions were used. Size is an important factor, for small samples may be soiled satisfactorily by a method that would not be at all adapted for larger samples of test cloth.

Few comments have been made by different investigators on the character of soiling achieved by a particular method employed. In the report of the work of the British Launderers' Research Association, Parker (28) states definitely that the method of soiling with Aquadag gives an even coating of graphite on the cloth. The color corresponds to "Grey II" and contains 0.0025 gm. of graphite per square foot. Grey II is one of the colors of the set of standard color disks made and used by the British Launderers' Research Association for measuring the tints of white goods in grading the work of power laundries. Soiling with Oildag, or with Oildag and olive oil, furnishes samples of color Grey II containing 0.08 gm. of Oildag or 0.08 gm. of Oildag with 0.08 gm. of olive oil per square foot after two treatments as described in Table 1 under formulas 3B and 3C, respectively. Heermann (14) admits that he did not succeed in producing uniform samples of even distribution of soil.

EXPERIMENTAL PROCEDURE AND CONCLUSIONS

STUDY OF THE DARK COMPONENT FOR THE SOILING MIXTURE

In developing a soiling mixture for use in this laboratory, tallow, olive oil, and a refined mineral oil were chosen as representative of the necessary fats and oils. Carbon tetrachloride was used as the medium when possible on account of its fat-dissolving power as well as its noninflammability. Since there is such a wide difference of opinion concerning the best dark constituent for such mixtures, this matter was given detailed study. Aquadag, Oildag, news ink, lamp-black, Cibacolor black B, and benzol soot were each considered with reference to uniform distribution after soiling and washing, the ease of removal of soil by the washing process, and the possibility of duplicating the brightness after soiling and washing by use of the same procedure.

In order to select the cotton fabric suitable for this work various kinds of plain woven cloth of different thread count and weight were soiled with an Oildag mixture and washed according to the procedure described (p. 546). A sheeting weighing 3.5 ounces per square yard and averaging 101 filling yarns and 106 warp yarns to the inch was found most satisfactory.

The cloth was desized by a method developed by the author and Peterson (29) in this laboratory. Four 18-inch squares of the desized sheeting were then weighed after conditioning overnight in a room maintained continuously at 70° to 75° F. and 50 per cent relative humidity.³ Three of the samples were next soiled simultaneously

³ Through the courtesy of E. O. Reed, the humidity room of the division of tests and technical control, U. S. Government Printing Office, was used for this work.

by agitating for 10 minutes in a small agitator type of washing machine in a suspension of the black constituent. The quantity and medium are indicated in Table 2. In addition, the samples were always treated in the same way with a solution of 30 gm. olive oil, 30 gm. tallow, and 60 gm. mineral oil in 4 liters of carbon tetrachloride. In experiments where Oildag, news ink, lampblack, and benzol soot were used the soiling was done in one application, since carbon tetrachloride was the medium for both the black constituent and the oils. With Aquadag and Cibonone black B, the black was applied in water, and after drying at room temperature, the samples were treated with the solution of oils in carbon tetrachloride.

TABLE 2.—*Brightness of samples soiled with various black substances, compared in a photometer with a milk-glass slide of 88.49 per cent brightness relative to magnesium carbonate (100 per cent)*

Experiment No.	Black constituent of soiling mixture	Quantity (grams)	Medium	Per cent brightness of—								Average per cent increase in brightness		Per cent brightness of unsoiled control				
				Soiled samples				Washed samples										
				Individual samples			Average	Individual samples			Average							
				A	B	C		Each run	Two runs	A		B	C	Each run	Two runs			
1	Aquadag (20 per cent graphite).	12.00	Water.	25.1	24.8	25.2	25.0	---	39.8	41.5	40.0	40.4	---	15.4	---	80.0	78.3	1.7
2	do.	---	---	24.9	25.7	25.0	25.2	25.1	41.1	40.4	39.5	40.3	40.4	15.1	15.2	80.0	78.1	1.9
3	Oildag (12.5 per cent graphite).	1.60	CCl ₄	27.2	27.4	26.7	27.1	---	53.6	54.1	51.7	53.1	---	26.0	---	81.5	79.5	2.0
4	do.	---	---	27.3	26.1	26.8	26.7	26.9	53.5	52.4	54.1	53.3	53.2	26.6	26.3	82.4	81.0	1.4
5	News ink (10 per cent carbon black).	.50	do.	27.2	26.8	27.5	27.2	---	47.1	46.6	47.3	47.0	---	19.8	---	81.4	80.6	0.8
6	do.	---	---	28.7	27.8	27.9	28.1	27.7	49.5	49.2	48.4	49.0	48.0	20.9	20.4	82.2	79.8	2.4
7	Lampblack	.25	do.	24.5	23.0	24.6	24.0	---	42.2	39.4	42.0	41.4	---	17.2	---	79.2	77.7	1.5
8	do.	---	---	23.3	22.9	23.1	23.1	23.6	40.9	41.3	42.4	41.5	41.4	18.4	17.8	79.5	77.7	1.8
9	Cibonone black B (10 per cent paste).	10.00	Water.	27.6	28.3	27.4	27.8	---	42.3	45.4	40.9	42.9	---	15.1	---	82.1	81.8	0.3
10	do.	---	---	29.9	29.1	27.9	29.0	28.4	46.3	44.8	45.0	45.4	44.2	16.4	15.8	82.4	82.2	0.2
11	Benzol soot	.15	CCl ₄	30.5	28.7	30.4	29.9	---	47.1	44.5	46.5	46.3	---	16.4	---	82.4	81.0	1.4
12	do.	---	---	28.4	27.7	27.9	28.0	29.0	42.7	42.0	42.5	42.4	44.4	14.4	15.4	82.5	81.4	1.1
Average				---				---	---				---	---		81.3	79.9	1.4

The samples were again hung in the humidity room to be weighed the following morning. Brightness values were then read on the soiled samples by means of an optical arrangement including a Martens photometer devised by Bruce (6) for the measurement of the hiding power of paints. These values were obtained by placing four thicknesses of the cloth over half of a milk-glass slide, which was used as a secondary standard and had a brightness of 88.49 per cent when calibrated against magnesium carbonate rated as 100 per cent. Four readings were made on each test sample, one approximately in the center of each quadrant. The cloth was so folded that the diagonal of the quadrant was parallel to the ends of the slide.

The washing procedure was the same throughout the experiment. The samples were washed separately in an experimental cylinder

washing machine maintained at constant temperature by a water bath. The cylinder revolved at 50 revolutions per minute, reversing its direction after each revolution. Every sample was washed three times for 10-minute periods in suds consisting of 4 liters of distilled water and 10 gm. of Castile soap, and was then rinsed three times for 10-minute periods in 4 liters of distilled water. A temperature of 75° C. was maintained throughout. There was no wringing during the process. After each bath the samples were removed and hung on the line until the next was prepared. A bank of two washers permitted two samples to be washed at the same time. Then the procedure was repeated with the third sample and the unsoiled control. The samples were dried at room temperature, conditioned overnight in the humidity room, and then weighed and their brightness determined by the photometer.

Table 2 gives the brightness values of the soiled and washed samples. Table 3 gives the percentages of soil on the test samples after soiling and after washing, as determined by weighing.

TABLE 3.—Percentage of soil on samples soiled with various black substances, determined by weighing under standard conditions of 70° to 75° F. and 50 per cent relative humidity, calculated on the basis of weight of unsoiled cloth

Ex- per- iment No.	Black con- stituent of soiling mixture	Per cent of soil on—										Average per cent of soil removed		Per cent in- crease in weight of un- soiled control after wash- ing
		Soiled samples					Washed samples							
		Individual samples			Average		Individual samples			Average		Each run	Two runs	
		A	B	C	Each run	Two runs	A	B	C	Each run	Two runs			
1	Aquadag	5.6	6.2	5.8	5.9	-----	3.2	3.4	3.2	3.3	-----	2.6	-----	0.32
2	do	5.7	6.0	5.6	5.8	5.8	3.0	3.2	3.0	3.1	3.2	2.7	2.6	.38
3	Oildag	4.6	4.7	5.2	4.8	-----	2.4	2.5	3.0	2.6	-----	2.2	-----	.17
4	do	4.8	4.8	4.4	4.7	4.8	3.2	3.6	3.1	3.3	3.0	1.4	1.8	.36
5	News ink	5.2	4.7	4.8	4.9	-----	3.5	3.2	3.4	3.4	-----	1.5	-----	.45
6	do	4.8	4.9	5.5	5.1	5.0	3.2	3.2	3.5	3.3	3.4	1.8	1.6	.21
7	Lampblack	5.0	5.4	4.7	5.0	-----	3.2	3.4	3.2	3.3	-----	1.7	-----	.51
8	do	5.4	5.7	5.2	5.4	5.2	3.1	3.2	3.1	3.1	3.2	2.3	2.0	.13
9	Cibanone black B.	5.5	4.8	4.9	5.1	-----	3.3	2.8	3.0	3.0	-----	2.1	-----	.15
10	do	5.1	5.4	5.2	5.2	5.2	3.6	3.8	3.1	3.5	3.3	1.7	1.9	.76
11	Benzolsoot	4.3	4.6	4.2	4.4	-----	3.2	3.4	3.3	3.3	-----	1.1	-----	.61
12	do	4.3	5.2	4.6	4.7	4.5	3.1	3.6	3.3	3.3	3.3	1.4	1.2	.25
	Average	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	.38

* 0.17 per cent represents decrease in weight and soils not included in the average 0.38.

According to the data in Tables 2 and 3 all the black constituents seem to fulfill the requirements of artificial soiling; that is, the soiling can be reproduced and the effect of washing can be duplicated in successive experiments. A second run in each set checked the first rather well. Samples soiled with Oildag were washed cleaner than any of the others, showing a 26.3 per cent brightness increase compared with a 20.4 per cent increase in the case of news ink and a 15 per cent approximate increase in the case of samples soiled with the other black substances. The unsoiled controls showed practically the same results throughout; that is, an almost insignificant decrease (1.4 per

cent) in brightness and, with the exception of experiment No. 3, a slight increase in weight with washing.

In order that the average brightness of the four quadrants may represent a reliable measure of the brightness of the entire surface of the test sample, good uniformity of depth of soil must exist throughout. The standard deviation was therefore calculated in order to obtain a measure of the variation of the brightness of each quadrant from the arithmetical mean. (Table 4.)

TABLE 4.—Average brightness value of six samples with two runs for each black constituent, and standard deviations and standard errors of averages

Black constituent of soiling mixture	Brightness of soiled samples (average of two runs)	Standard deviation	Standard errors $\sigma M = \frac{\sigma}{\sqrt{N}}$ $N=24$	Brightness of washed samples (average of two runs)	Standard deviation	Standard errors $\sigma M = \frac{\sigma}{\sqrt{N}}$ $N=24$
	<i>Per cent</i>			<i>Per cent</i>		
Aquadag.....	25.1	0.63	0.13	40.4	1.34	0.27
Oildag.....	26.9	.66	.14	53.2	1.09	.22
News ink.....	27.7	.85	.17	48.0	1.48	.30
Lampblack.....	23.6	1.02	.21	41.4	1.86	.38
Cibanone black B.....	28.4	1.21	.25	44.2	2.56	.52
Benzol soot.....	29.0	1.39*	.28	44.4	2.21	.45

* σM equals standard error of the mean; σ equals standard deviation; and N equals number of observations.

Obviously the black constituent showing the smallest standard deviation is more highly recommended than the others for use in the artificial soiling mixture. In both soiled and washed samples, the black substances permanently suspended when purchased, exclusive of Cibanone black B, have smaller standard deviations than the dry carbons—lampblack and benzol soot. Of the permanently suspended blacks, Aquadag and Oildag are superior to news ink (carbon black) as regards uniformity within each sample and in respect to their ability to soil three samples uniformly and simultaneously in the same run. Oildag is to be preferred to Aquadag because it can be attached to the fabric together with oils in one application.

Table 4 reveals further that the soiled samples as a group show less variation than the washed samples with every black component. Even soiling is apparently less difficult than even washing with the apparatus used in these experiments.

The reliability of the brightness increase figure, which is an index of washing effect, is of great importance. Since no correlation exists between brightness of spots on soiled and washed samples, respectively, in the close range dealt with, the reliability of this figure can be determined by the formula

$$\sigma d = \sqrt{\frac{\sigma s^2 + \sigma w^2}{N_r}}$$

where σd equals standard error of difference, σs equals standard deviation of the soiled samples, σw equals standard deviation of the washed samples, and N_r equals the number of brightness readings made on both soiled and washed samples. N_r equals 24 in all calculations, since four readings are made on each sample and there are six samples in two runs. The value indicating the increase in brightness together with the standard deviation for Oildag is 26.3 ± 0.26 , which

means that chances are about two to one that the brightness increase with two runs, three samples each, four brightness readings on each sample, would not vary from the 26.3 by more than 0.26. By contrast with the reliability of the brightness increase figure for Oildag, benzol soot shows 15.4 ± 0.53 , and Cibanone black B, 15.8 ± 0.58 . Oildag would be selected again because of the greater reliability exhibited by the figure expressing brightness increase.

METHOD OF APPLYING THE SOILING MIXTURE TO THE TEST CLOTH

The selection of a suitable method of applying the soiling mixture involved the study of a number of mechanical devices. It was found that the use of a ball mill, a laboratory mechanical stirrer, and an oscillating shaking machine were not satisfactory on account of the irregular soiling produced. Of these the oscillating shaking machine was most promising.

Better results were produced by placing the soiling mixture in a shallow pan and dipping the sample into it. However, it was found necessary to use a strictly uniform procedure if comparable results were to be obtained. The best method of doing this consisted in immersing the sample for one minute, reversing it, and allowing it to remain immersed for another minute. It was then lifted out and turned once while drying. The distribution of the soil over the surface of the samples was fairly good. The duplicating of the brightness of soil with a second trial was poor with the lampblack constituent but excellent with the Oildag.

Another method, which was unsuccessful but which undoubtedly has possibilities, consists in stretching the fabric over frames which are then fitted into the grooves of a photographic tank. By blowing air into the bottom of this, the soiling mixture can be circulated and kept from settling during the half-hour exposure of the test samples.

The procedure finally selected for all the soiling done in this laboratory involved the use of a small agitator washing machine. In the bottom of the tub is a four-bladed aluminum agitator which is operated by a hand lever. The revolving device is reversed after each revolution and thus carries the cloth first in one direction and then in the other. By use of this machine it has been possible to soil each test sample uniformly, to soil three pieces similarly at the same time, and to duplicate a soiling of three samples with the same treatment.

QUANTITATIVE DETERMINATION OF WASHING EFFECT

Bergell (4), Lindner (23, 24), Parker (28), and Schiewe and Stiepel (30) have suggested that the amount of soil removed from samples in experimental laundering studies may be determined by weighing, although Heermann (14) specifically advises against this method. In order to test the feasibility of this suggestion the two standard methods of weighing, in a constant humidity room and in a conditioning oven, were compared. Photometric readings and weighings in a constant humidity room had been made on all samples up to this point.

TABLE 5.—*Brightness of Oildag-soiled samples, unheated and heated, compared in a photometer with a milk-glass slide of 88.49 per cent brightness relative to magnesium carbonate (100 per cent)*

Experiment No.	Black constituent of soiling mixture	Per cent brightness of—										Average per cent increase in brightness	Per cent brightness of unsoiled control				
		Soiled samples					Washed samples										
		Individual samples			Average	Individual samples			Average								
		A	B	C		Each run	Two runs	A		B	C					Each run	Two runs
3	Oildag, unheated	27.2	27.4	26.7	27.1	—	53.6	54.1	51.7	53.1	—	26.0	—	81.5	79.5	—2.0	
4	do	27.3	26.1	26.8	26.7	26.9	53.5	52.4	54.1	53.3	53.2	26.6	26.3	82.4	81.0	—1.4	
13	Oildag, heated	30.3	29.6	29.4	29.8	—	34.2	34.1	34.9	34.4	—	4.6	—	82.1	81.3	—0.8	
14	do	30.8	30.6	29.7	30.4	30.1	36.0	35.5	34.8	35.4	34.9	5.0	4.8	81.4	81.5	—0.1	

The samples in the experiment with heated Oildag (Table 5, experiments 13 and 14) were subjected to the same soiling and washing procedures as those with unheated Oildag (Table 5, experiments 3 and 4). The only difference was that, in the former, weighings were made in each case with the samples in a bone-dry condition. This was accomplished by heating in a conditioning oven at 105° to 110° C. for four hours with a fan running. Brightness values of the samples are recorded in Table 5 and the weighings in Table 6.

TABLE 6.—*Percentage of soil on Oildag-soiled samples, unheated and heated, determined by weighing in a room maintained at 70° to 75° F. and 50 per cent relative humidity, and in a conditioning oven at 105° to 110° C., respectively, calculated on the basis of weight of unsoiled cloth*

Ex- per- iment No.	Black constit- uent of soil- ing mixture	Per cent soil on—										Average per cent of soil removed		Per cent differ- ence in weight of un- soiled control after wash- ing
		Soiled samples					Washed samples							
		Individual sam- ples			Average		Individual sam- ples			Average				
		A	B	C	Each run	Two runs	A	B	C	Each run	Two runs	Each run	Two runs	
3	Oildag, un- heated	4.6	4.7	5.2	4.8	-----	2.4	2.5	3.0	2.6	-----	2.2	-----	-0.17
4	do	4.8	4.8	4.4	4.7	4.8	3.2	3.6	3.1	3.3	3.0	1.4	1.8	+0.36
13	Oildag, heat- ed	4.5	4.4	4.8	4.6	-----	2.8	2.8	2.8	2.8	-----	1.8	-----	-0.12
14	do	4.5	4.6	5.0	4.7	4.6	2.7	2.9	3.1	2.9	2.8	1.8	1.8	-0.04

A comparison of the average percentage brightness increase of the three samples in each set shows a 26 per cent approximate increase in the unheated against a 5 per cent increase in the heated samples. Mere visual evaluation with the naked eye agrees with this finding, and yet weighings show 1.8 per cent of soil removed in each case. Here, as also in the humidity room weighings, very poor correlation exists between brightness values and percentage soil on the weight of the cloth. Descending brightness values should give ascending per-

centages representing soil on the weight of the cloth. The two standard methods of weighing textile fabrics fail to be useful in determining the results of laundering experiments. The humidity room permits weighing under standard conditions but requires too long a time for the samples to reach equilibrium. Jarrell (22) finds that 48 hours are required for the attainment of equilibrium when paper is transferred from 35 per cent to 65 per cent relative humidity and about 6 days when changed from 65 per cent to 35 per cent. In contrast to this the conditioning oven furnishes speed but is also unreliable as a method of determining true experimental results. In addition, the soil is apparently fastened to the fabric so that washing effects can not be studied.

A photometric method was the only other used in this laboratory for determining the amount of soil removed. Bruce's (6) arrangement of Martens photometer is reasonably satisfactory for this purpose in that it permits any desired number of readings over the surface of the cloth. The great disadvantage lies in the small area that each brightness reading represents. This is only one-half of a circle 3 cm. in diameter. In the writer's experiments the soil has been evenly enough distributed to allow an average of four readings, one in each quadrant, to represent the reflection value of the entire surface of the 18-inch square. However, an instrument may be arranged to read a larger surface of the test sample. Brightness readings are preferred to weighings as a method of determining the results of the washing process.

SUMMARY

A comparative study of the black constituents suggested for use in experimental soiling methods results in the recommendation of Oildag for this purpose. Samples soiled with mixtures containing this material are of uniform and reproducible brightness.

The cotton sheeting used in these experiments was of suitable yarn composition and weave to permit a uniform distribution of the dirt mixture after soiling and after washing.

The soiling mixture now being used by this bureau in experimental laundering studies contains the following constituents: Oildag, olive oil, tallow, and mineral oil. Representatives of all constituents of natural soiling are present with the exception of albuminous matter and stains. Albuminous matter, if included, would limit the temperature range of experimental work.

A small agitator type of washing machine is used in applying the mixture to the fabric. It deposits the soil uniformly over the surface of each sample, furnishes samples of approximately the same brightness, and with the same procedure enables the production of a second set of the same uniformity and brightness.

Weighing, either in a humidity room or in a conditioning oven, was found to be an unsatisfactory method of determining the amount of soil removed. A photometric method is therefore now being employed.

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THE CHEMICAL COMPOSITION OF GIRASOLE AND CHICORY GROWN IN MINNESOTA¹

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INTRODUCTION

In connection with the utilization of the girasole, or Jerusalem-artichoke, *Helianthus tuberosus*,⁴ and chicory, *Cichorium intybus*, as truck crops and in possible sugar manufacture, the chemical composition is of interest. In the case of the girasole a knowledge of the composition of the tops is important because of the value of this portion of the plant as a food for stock. In the present paper are reported analyses of the crops grown at University Farm, St. Paul, Minn., in 1927.

The material was analyzed according to the Official Methods (1)⁵ except the sugars in girasole tubers and chicory roots. These were determined by Ost's cupro-carbonate method as modified by Nyns⁶ and reviewed by Oliver,⁷ and Traub, Thor, Willaman, and Oliver.⁸

GIRASOLE

Analyses of the various constituents of girasole grown under European conditions have been reported by König (8, v. 1, p. 729; v. 2, p. 900-901), Fingerling-Kellner (7), and by Müntz and Girard as quoted by Garola (4). The present analyses show the composition of girasole under Minnesota conditions. For these analyses four varieties were chosen—Portland, Mammoth French white, a purple variety, and a variety sent out by the United States Department of Agriculture, here referred to as U. S. D. A. The plants were grown on rich sandy loam. A comparison of the composition of tubers of the four varieties are presented in Table 1.

In these tubers there is, generally speaking, a rise in the dry matter, carbohydrate fractions, and total nitrogen from August 30 to November 3. On the latter date the highest total sugar content was a little more than 15 per cent in the Portland and Mammoth French white varieties. The sugar content of the U. S. D. A. and purple varieties was slightly lower.

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⁴ *Helianthus tuberosus* is not an artichoke and is not native to Palestine; therefore "Jerusalem-artichoke" is a misnomer, and is being replaced by the simpler term "girasole." See L. H. Bailey (2, p. 446-449).

⁵ Reference is made by number (italic) to "Literature cited," p. 555.

⁶ NYNS, L. SELECTIVE DETERMINATION OF LEVULOSE BY MEANS OF OST'S CUPRO-CARBONATE SOLUTION. Chem. Abs. 19: 1236. 1925.

⁷ OLIVER, R. THE DEVELOPMENT OF METHODS FOR THE ANALYSES OF INULIN BEARING PLANTS. 1927. [Unpublished master's thesis. Copy on file, Univ. Minn. Libr.]

⁸ TRAUB, H. P., THOR, C. J., WILLAMAN, J. J., and OLIVER, R. STORAGE OF TRUCK CROPS. THE GIRASOLE *HELIANTHUS TUBEROSUS*. Plant Physiol. 4: 123-134, illus. 1929.

The important consideration in the manufacture of fructose is the ratio of fructose to total sugars (6). In the ratio of fructose to other sugars present Mammoth French white ranks highest, followed by U. S. D. A., Portland, and the purple variety, in the order named.

To determine the relative value of the various parts of the whole plant, analyses were made of the tubers, leaves, and stems of one variety. The data covering the composition of tubers, leaves, stems, and entire tops of the Portland variety are summarized in Table 2.

TABLE 1.—*Composition of the tubers of four girasole varieties grown on sandy loam at University Farm, St. Paul, Minn., and analyzed August 30 and November 3, 1927*

Variety	Date analyzed	Composition expressed as percentage of green weight							
		Moisture	Dry matter	Fructose	Glucose	Total sugar	Ash	Crude protein	Crude lipides*
Portland.....	{Aug. 30	84.6	15.3	6.72	3.61	10.33	1.09	1.55	0.26
	{Nov. 3	79.8	20.1	9.99	5.06	15.05	1.08	2.56	-----
Mammoth French white.....	{Aug. 30	83.5	16.4	7.52	3.43	10.95	1.24	1.75	.24
	{Nov. 3	79.1	20.8	10.3	4.79	15.09	1.13	2.43	.17
U. S. D. A.....	{Aug. 30	84.6	15.3	7.29	3.72	11.01	1.00	1.50	-----
	{Nov. 3	80.2	19.7	9.33	4.43	13.96	1.16	2.62	-----
Purple.....	do.	78.9	21.0	9.38	4.74	14.12	1.18	3.06	-----

Variety	Date analyzed	Composition expressed as percentage of dry weight						Ratio of—	
		Fructose	Glucose	Total sugar	Ash	Crude protein	Crude lipides	Fructose to glucose	Fructose to total sugar
Portland.....	{Aug. 30	43.6	23.4	67.1	7.0	10.0	1.6	1.86	0.65
	{Nov. 3	49.5	25.0	74.6	5.3	12.7	-----	1.97	.66
Mammoth French white.....	{Aug. 30	45.8	20.9	66.7	7.5	10.5	1.4	2.19	.69
	{Nov. 3	49.4	22.9	72.3	5.4	11.6	.8	2.15	.68
U. S. D. A.....	{Aug. 30	47.4	24.2	71.6	6.5	9.7	-----	1.96	.66
	{Nov. 3	48.3	22.4	70.7	5.8	13.3	-----	2.15	.68
Purple.....	do.	44.6	22.5	67.1	5.6	15.6	-----	1.98	.66

* Chloroform extract.

TABLE 2.—*Chemical composition of tubers, leaves, and stems of girasole plants of the Portland variety, grown at University Farm, St. Paul, Minn., in 1927*

Determination	Percentage of green weight in—				Percentage of dry weight in—			
	Tubers ^a	Leaves ^b	Stems ^b	Entire top ^b	Tubers ^a	Leaves ^b	Stems ^b	Entire top ^b
Moisture.....	79.8	75.8	66.1	69.9	-----	-----	-----	-----
Dry matter.....	20.1	24.1	33.8	30.0	-----	-----	-----	-----
Reducing sugars.....	-----	1.72	1.96	1.86	-----	7.14	5.79	6.22
Nonreducing sugars.....	-----	2.03	6.03	4.44	-----	8.37	17.81	14.79
Total sugars.....	-----	3.75	7.99	6.30	-----	15.51	23.60	21.00
Starch.....	-----	1.02	6.25	4.18	-----	4.21	18.47	13.91
Pentosans.....	.83	2.07	.42	1.07	3.90	8.56	1.25	3.59
Protein (×6.25).....	2.56	3.75	.93	2.00	12.75	15.43	2.81	0.87
Ash.....	1.08	3.58	.74	1.87	5.37	14.80	2.18	6.21
Crude lipides (chloroform extract).....	-----	.17	.04	-----	-----	.07	.12	-----

^a Nov. 3.

^b Oct. 11.

* Total water-soluble carbohydrates.

The dry matter in the leaves, stems, and entire tops of the girasole is relatively higher than that in the tuber. The tubers contain relatively much more sugar than the leaves, stems, and entire top. The starch content of the leaves, determined by the diastase method, is relatively lower than that of the stem portion. The pentosan, nitrogen, and ash content of the leaves is relatively much greater than that of the stem. In the case of the tubers, the pentosan value was determined on the residue of a sample after thorough extraction with 80 per cent alcohol and hot water. The amount recovered is the same as that reported by Müntz and Girard (4).

The total sugar content of the entire top is a little more than 6 per cent of the green weight and 21 per cent of the dry weight. The starch content is more than 4 per cent of the green weight and more than 13 per cent of the dry weight. The protein content is 2 per cent of the green material and more than 6 per cent of the dry material. In the utilization of the tops for feeding and other purposes these facts are important.

A comparison of the Minnesota analyses with those of Müntz and Girard (4) in Europe has been attempted in Table 3. The table shows that girasole tubers and tops grown under Minnesota conditions compare favorably with those grown in Europe.

TABLE 3.—Chemical composition of mature girasole tubers, tops, and leaves, as determined by the writers and by Müntz and Girard (4)

[Values are expressed in percentage of green weight]

Determination	Composition of tubers as shown by—		Composition of top as shown by—		Composition of leaves as shown by—	
	Present data	Müntz and Girard	Present data	Müntz and Girard	Present data	Müntz and Girard
Moisture.....	79.8	81.9	69.9	83.0	75.8	84.2
Dry matter.....	20.1	18.1	30.0	17.0	24.1	15.8
Reducing sugars.....	(^a)		1.86		1.72	
Nonreducing sugars.....			4.44		2.03	
Total sugars.....	^b 15.04	^c 11.05	6.30		3.75	
Starch.....			4.18		1.02	
Pentosans.....	.83	.83	1.07	1.99	2.07	1.39
Protein.....	2.56	2.46	2.00	2.54	3.75	3.08
Ash.....	1.08	1.50	1.87	2.89	3.58	3.31

^a Blanks indicate that no figures were reported.

^b Total water-soluble carbohydrates.

^c Inulin plus sugar.

Typical acre yields of girasole tubers of the Portland variety for the season 1927 in Minnesota, based upon $\frac{1}{6}$ -acre plots on rich sandy loam and silt loam, are, for sandy loam, 11.50 tons per acre, and for silt loam 5.25 tons per acre. The crop on the sandy loam was planted the middle of April, and that on silt loam the first week in May, and this difference in date of planting may account in part for the great variation in yield on the two soils. These yields compare favorably with those reported for France by Shoemaker (9). On October 1 the plants on silt loam averaged 7½ feet in height and were just, beginning to flower; on sandy loam on the same date the plants averaged 8 feet and were in full bloom.

CHICORY

Analyses of chicory have been reported by Tatlock and Thomas (11) and others who have studied chicory as a substitute for coffee. Colin (3), Sirot and Joret (10), and Grafe and Vouk (5) have reported on inulin in chicory. These analyses are not strictly comparable with those reported in this paper, where chicory is considered as a source of fructose.

TABLE 4.—Comparison of the chemical composition of the roots of two varieties of chicory grown at University Farm, St. Paul, Minn., and analyzed September 15 and October 14, 1927

Variety	Date analyzed	Composition in percentage of green weight						
		Moisture	Dry matter	Fructose	Glucose	Total sugar	Ash	Crude protein
Large-rooted Magdeburg.....	Sept. 15	81.7	18.2	10.13	3.53	13.66	0.72	1.25
	Oct. 14	79.4	20.5	10.58	5.99	16.57	.73	1.37
Westland's strain.....	Sept. 15	80.6	19.3	10.68	3.47	14.15	.79	1.20
	Oct. 14	77.6	22.3	10.79	6.63	17.42	.71	1.62

Variety	Date analyzed	Composition in percentage of dry weight					Ratio of —	
		Fructose	Glucose	Total sugar	Ash	Crude protein	Fructose to glucose	Fructose to total sugar
Large-rooted Magdeburg.....	Sept. 15	55.47	19.33	74.80	3.96	6.8	2.87	0.74
	Oct. 14	51.51	29.16	80.67	3.57	6.8	1.76	.64
Westland's strain.....	Sept. 15	55.25	17.95	73.20	4.08	6.1	3.08	.75
	Oct. 14	48.23	29.64	77.87	3.19	7.3	1.63	.62

• On a green-weight basis.

A comparison of two superior varieties of chicory, Large-rooted Magdeburg and Westland's strain, grown on silt loam at University Farm, St. Paul, Minn., is presented in Table 4. This table shows that the increase in dry matter between September 15 and October 14 amounts to 2.3 and 3.0 per cent of the green weight for Large-rooted Magdeburg and Westland's strain, respectively. The table shows also that there is a perceptible increase in total sugars in both varieties from September 15 to October 14, amounting to about 3 per cent of the green weight. This difference is due to a relatively greater increase of sugar other than fructose, since the latter value remains practically constant. The absolute amount of water-soluble carbohydrates in both varieties at maturity, October 14, is practically the same, 16.57 and 17.42 per cent, of the green weight. The fructose values for these two varieties are 10.58 and 10.79 per cent of the green weight. The ash content for both varieties is about 0.7 and the protein content about 1.2 per cent.

In connection with the ratios of fructose to glucose and fructose to total sugars, on a green-weight basis, it should be noted that the highest ratios are reached at the earlier date, September 15. By October 14, because of the relative increase in glucose, the ratios decrease markedly.

COMPARISON OF GIRASOLE AND CHICORY

A comparison of the fructose and total sugar content of chicory on September 15 and girasole at its best on November 3 shows that, under Minnesota conditions, chicory has consistently a higher fructose and total sugar content. The ratios of fructose to glucose and fructose to total sugars are also higher for chicory. (Tables 1 and 4.) These considerations are important in the possible utilization of chicory as a source of fructose.

SUMMARY

Two of the best varieties of the girasole grown, Portland and Mammoth French white, contain a little more than 15 per cent sugar on a green-weight basis.

Mammoth French white has the higher ratio of fructose to total sugars.

The sugar content of the tubers is relatively greater than that of the tops.

The total sugar and starch content of the tops is more than 10 per cent of the green and more than 30 per cent of the dry weight.

As a source of fructose, chicory is apparently as desirable as, or more desirable than, girasole.

Chicory apparently reaches its maximum fructose content relatively earlier (September in Minnesota) than girasole (November in Minnesota).

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MOSAIC DISEASES IN THE CANARY ISLANDS, WEST AFRICA, AND GIBRALTAR¹

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INTRODUCTION

The Allison V. Armour expedition was conducted primarily for the purpose of collecting living plants and seeds and introducing them into the United States. However, as the yacht used on the expedition was fitted for doing laboratory work and for growing potted plants, it was possible to conduct certain types of experiments coincident with the work of collection; and other experiments have been conducted since the return of the writer to the United States. On the expedition the writer's time was devoted to the search for plant viruses suitable for experimental studies and to the accumulation of information concerning the virus diseases of plants in the regions visited.

This report deals with the observations made in the Canary Islands, west Africa, and Gibraltar, and with some of the experimental results relating both directly and indirectly to the mosaic viruses from *Nicotiana glauca* R. Grah. collected on the Canary Islands and at Gibraltar. Limited comparative studies have been made with some of the viruses collected in the United States.

MOSAICS IN CANARY ISLANDS

The expedition reached Santa Cruz, Teneriffe, the capital of the Canary group, on December 22, and on December 28 it went to the island of Grand Canary. Several days were spent making observations and collections on each of these islands. During December the islands are rather cool and are rich in growing plants, including many kinds of garden vegetables.

Tomatoes and potatoes were growing in many localities, and various species of cucurbits were found in gardens and along the roadsides. Many of the potato plantings were located on narrow terraces, sometimes high above the highways and very inaccessible. In one of these terrace plantings which overlooked the sea near the port of Orotava, Teneriffe, green mosaic was found on most of the potatoes and on a garliclike plant³ (fig. 1) which was growing as a

¹ Received for publication Mar. 2, 1929; issued October, 1929. The work described in this paper was done by the Allison V. Armour expedition of 1923-27. A full account of the two expeditions sponsored by Armour has been published by Fairchild (7).⁴

² The writer wishes to express his appreciation to Mr. Armour and to Doctor Fairchild for the opportunity of collecting and studying the mosaic diseases reported in this paper and for personal assistance rendered in various ways during the voyage. Credit is due to Dr. J. M. Dalziel, of Kew Botanical Gardens, Kew, England, who accompanied the expedition, and also to Oliver M. Freeman, of the United States Bureau of Plant Industry, for identifying some of the plants collected.

³ When this plant was collected it was thought to be a wild garlic, and the writer referred to it as such in an abstract (6). After being studied further in a quarantine greenhouse, this plant appears to be one of the false garlics (*Nothoscordum fragrans* Kunth).

⁴ Reference is made by number (italic) to "Literature cited," p. 577.

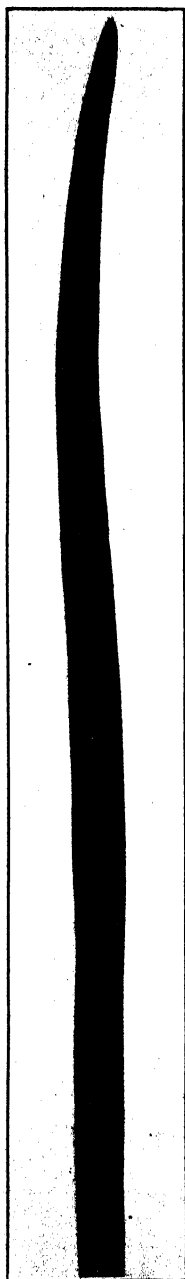


FIGURE 1.—Green mosaic on false garlic (*Nahoscordum fragrans* Kunth). Collected on the island of Tenerife, Canary Islands. $\times 1$

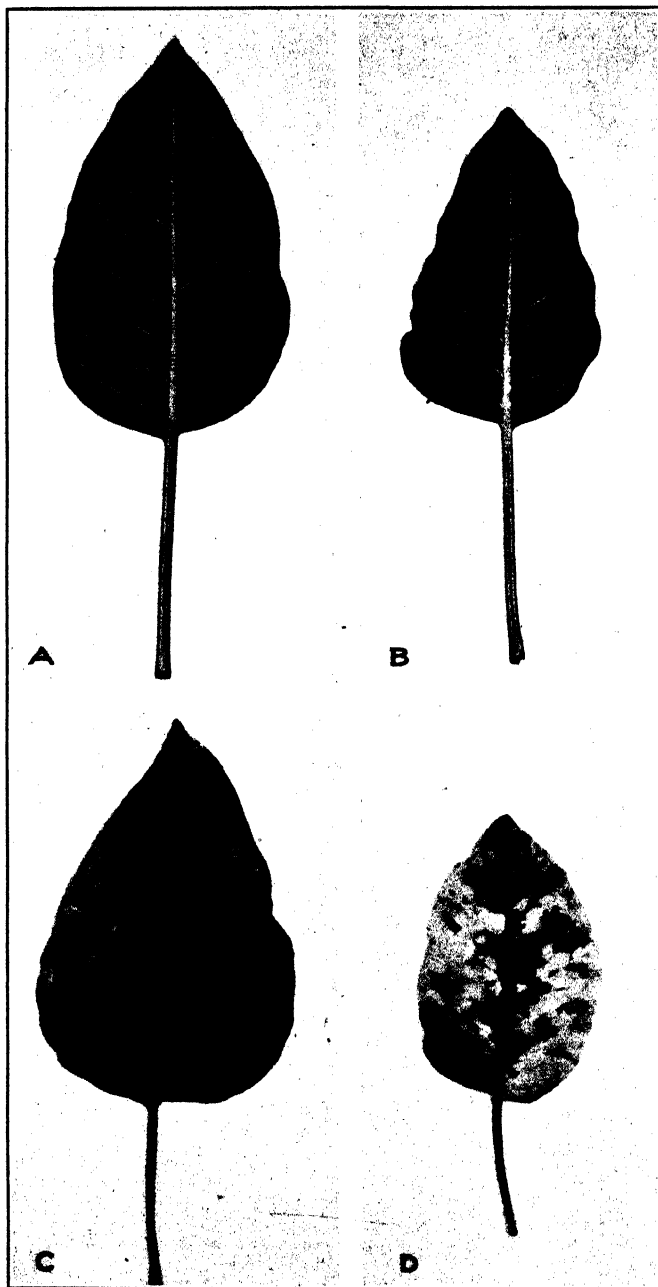


FIGURE 2.—Mosaic on *Nicotiana glauca*: A, Mosaic-free leaf; B, mild mosaic; C, light-green mosaic; D, yellow mosaic. Collected on the island of Grand Canary, Canary Islands. $\times 1$

weed among the potatoes. In one garden at Orotava, fuchias (*Fuchsia magellanica gracilis*) and cultivated peppers (*Capsicum frutescens* L. var. *grossum*) had typical green mosaic. Many tomato plants were examined on the islands, but no mosaic was observed. Also no mosaic was observed on the cucurbit species which were found.

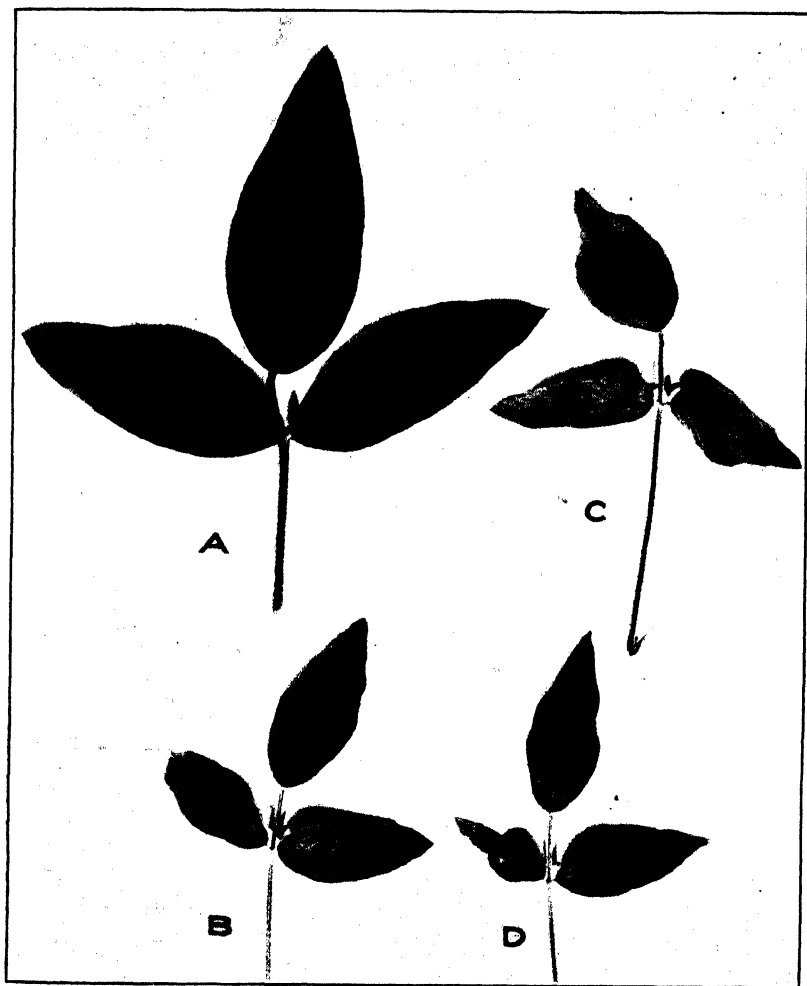


FIGURE 3.—Green mosaic on *Psoralea bituminosa*: A, Healthy leaf; B-D, diseased leaves. Collected on the island of Tenerife, Canary Islands. $\times \frac{1}{16}$

Bananas are grown in all suitable areas on both of the islands. Many plantations were visited and hundreds of plants were examined for bunchy top, a destructive virus disease (7) which occurs in Australia, Fiji, Egypt, Ceylon, and the Philippine Islands. None of the plants examined gave evidence of this disease, and no mosaic was observed.

Psoralea bituminosa L., a legume which is a native of Arabia and the Mediterranean districts, and *Nicotiana glauca*,⁵ a native of South America, were found growing wild in all the waste places and barrancos or dry creek channels. Both species had green mosaic. (Figs. 2 and 3.) In fact, it was exceptional to find mosaic-free plants of either of the species on Tenerife or Grand Canary.

EXPERIMENTS WITH THE CANARY ISLANDS MOSAICS

The mosaic on *Nicotiana glauca* was of greatest immediate interest, and several collections of diseased tissue were made. However, these could not be tested until young tobacco plants were available on the expedition yacht.

It was found that Connecticut-Havana tobacco developed very satisfactorily on a special deck arranged near the stern of the boat.

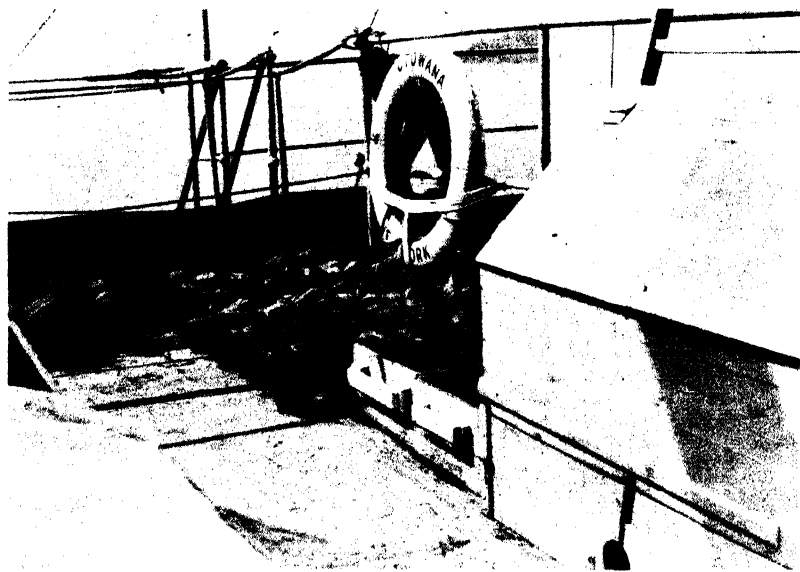


FIGURE 4.—Method of growing experimental tobacco plants on the deck of the yacht *Utowana*

(Fig. 4.) As no serious difficulty was experienced from storms, it was possible to grow the plants in the open. The intense light from the tropical sun and any salt-water spray were kept from the plants by means of properly arranged cheesecloth and canvas. The plants were grown in 4-inch pots arranged in a large meshed net made of heavy cord and wire. This prevented the pots from rolling about.

An inoculation test was made with an extract from dried leaf tissue obtained from a mosaic-affected plant of *Nicotiana glauca* collected in Tenerife. This mosaic was easily transmitted to tobacco. From the beginning, the symptoms were of the light-green type. After the plants had developed 12 to 18 leaves, yellow spots and

⁵ This species grows wild in many districts in the southwestern part of the United States, and mosaic-diseased specimens have been sent to the writer by David Fairchild from the Boyce Thompson Southwestern Arboretum at Superior, Ariz.

patches appeared on some of the foliage, as indicated in Figure 5, A. These results were of especial interest as they are similar to those that had been obtained with all the mosaic viruses that had been tested on tobacco previously (4, 5).

Another test was carried out with virus from *Nicotiana glauca* collected in Grand Canary. The green-mosaic symptoms were slightly different from those obtained in the previous experiments, but yellow spots occurred in the same manner.

When the ship returned to the Canary Islands for fuel in March, one day was spent collecting and observing mosaic-affected *Nicotiana glauca* growing in the regions of the upper and lower highways between Las Palmas and Telde on the island of Grand Canary. Several hundred diseased plants were examined, and of these one had what



FIGURE 5.—Mosaic on Connecticut-Havana tobacco: A, Light-green mosaic showing a yellow spot; B, yellow mosaic produced by a concentrated virus obtained from spots similar to that shown in A; C, mild dark-green mosaic obtained from *Nicotiana glauca*. $\times \frac{1}{3}$

appeared to be pure yellow mosaic (fig. 2, D) and two had a yellow and green combination with yellow predominating. The remainder had either a light-green (fig. 2, C) or a mild dark-green mosaic (fig. 2, B). In some cases leaf abnormality was associated with green mosaic.

Mosaic leaf tissue was collected from eight specimens which had somewhat different symptoms ranging from the mild dark-green mosaic to the yellow type. These tissues were handled with great care to prevent cross contamination after collection. The fresh leaves were cut into fine pieces and dried in a warm closet, after which they were carefully stored in envelopes separated from each other by means of heavy oiled paper.

All these dry tissues have been tested on a strain of Connecticut-Havana tobacco in a quarantine greenhouse, and each produced mosaic one year after collection. Ten or more plants were inocu-

lated with each sample. Plants inoculated with virus from different sources were protected from one another by means of frames having galvanized wire screen (12 meshes to the inch each way) tacked on each side, as shown in Figure 6. A frame of single screen was placed in front of the plants. Extensions were arranged on top of the partition screens if the plants became very tall. All openings in the greenhouse were covered with copper screen having 30 wires to the inch each way. The legs of the greenhouse benches were coated with "tanglefoot" paste. The benches did not touch the walls of the house at any point.

Thus far it seems that the mottling symptoms produced by these viruses on tobacco fall into three general classes: (1) Yellow mosaic, a type which has little or no chlorophyll in the lightest-colored areas; (2) light-green mosaic, a type characterized by light-green areas which occupy the greatest portion of the leaf surface, normal dark-



FIGURE 6.—Method used in conducting greenhouse experiments with different viruses. One front screen was removed for the purpose of making the photograph

green areas, and occasional yellow spots; and (3) mild dark-green mosaic, a type in which the dark or normal green is usually in excess of the light-green portion of the leaf, and yellow spots occur rarely. In young plants mild dark-green mosaic is very inconspicuous.

The yellow mosaic, thought to be pure when collected, proved not to be free from the green type. In fact, it appears that the three viruses were present in some of the diseased plants collected. All the green mosaics collected have been studied on maturing tobacco plants, and all have produced yellow spots. Some of the light-green types developed yellow spots in all the inoculated plants. One of the mild dark-green forms has produced yellow spots on 2 of the 52 plants which have been inoculated.

When yellow spots were removed from mosaic-affected leaves and inoculated into young plants, a yellow type of mosaic developed soon after the inoculation. (Fig. 5, B.) By making several successive subinoculations from the increasing number of yellow spots, strains of concentrated virus of yellow mosaic have been developed. From

three to seven isolations and subinoculations have been required to accomplish this. Figure 7 shows a plant inoculated with such a concentrated virus. The yellow mosaics isolated thus far from *Nicotiana glauca* are similar in appearance to the yellow mosaic isolated by the writer (4) from cultivated tobacco having light-green mosaic.

Yellow mosaics are very attractive and ornamental, but they are more destructive than the green types. When they are inoculated into tomato plants, a high percentage of killing frequently results. In some cases young tobacco plants have been killed, and in all cases tobacco plants are markedly stunted, and the lower leaves become very yellow and frequently are killed completely.

One of the outstanding characteristics of the viruses of yellow mosaic is their ability to produce a general chlorosis on the old foliage.

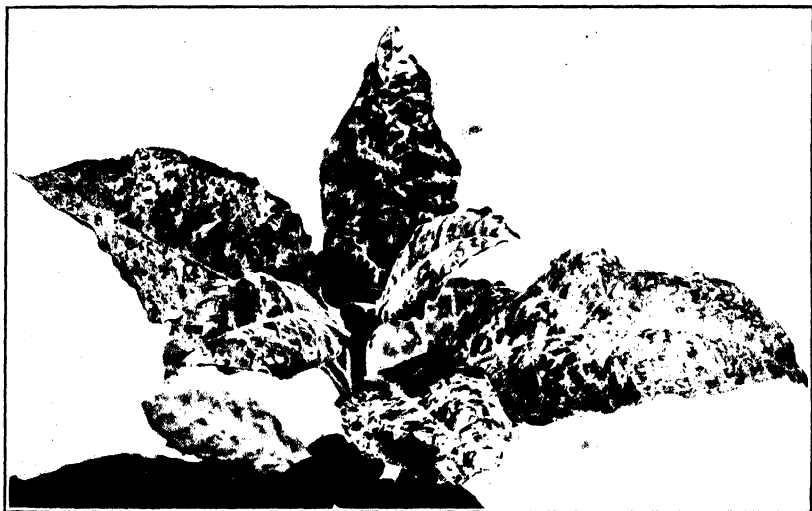


FIGURE 7.—Yellow mosaic on Connecticut-Havana tobacco. The virus used was concentrated by isolating yellow spots from tobacco leaves (fig. 5, A) infected with light-green mosaic induced from virus collected on *Nicotiana glauca*. $\times \frac{1}{4}$

Tobacco plants 30 inches tall at the time of inoculation have developed chlorosis of the type shown in Figure 8 on all the leaves which were over 3 inches in length at the time of inoculation.

The yellow mosaic pattern on the older leaves is quite distinct from that which occurs on the young leaves (fig. 7), and it is very distinct from the yellowing associated with senility. This chlorotic pattern occurs in a milder form on detached leaves which are inoculated with the virus of yellow mosaic and cultured in sand according to the methods of Purdy (9). Mottling is very pronounced on the stems and fruit of tobacco plants having yellow mosaic. (Figs. 9 and 10.)

Many combinations of yellow and green mosaic symptoms have been produced by inoculating tobacco and tomato plants with extracts consisting of different proportions of viruses from yellow mosaic and light-green mosaic plants. Several of these synthetic combinations resembled yellow and green mosaics which the writer ob-

served on *Nicotiana glauca* in the Canary Islands, and on tomato plants in the southern United States.

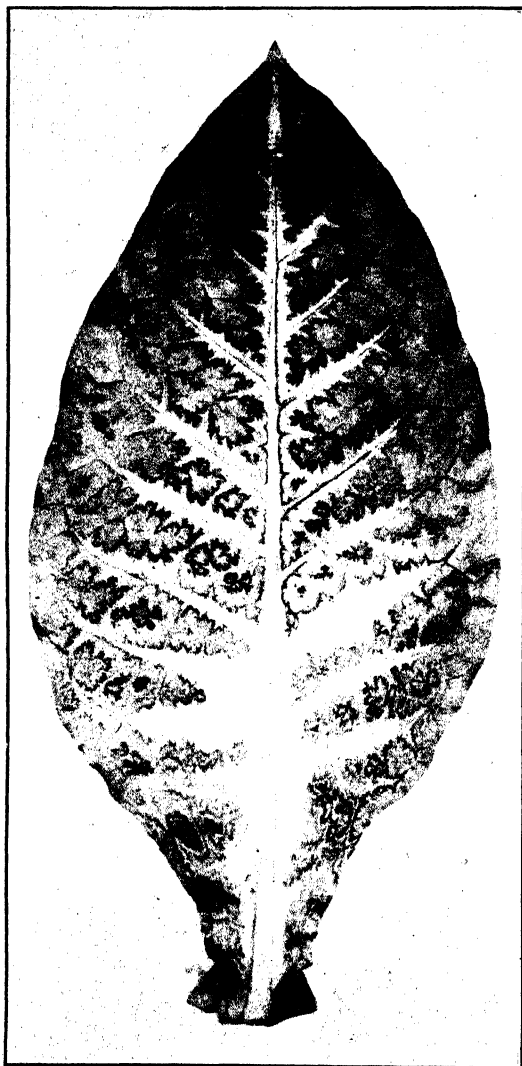


FIGURE 8.—Mature leaf of Connecticut-Havana tobacco. This leaf was about 3 inches long and the plant was 36 inches tall at the time of inoculation with a virus of yellow mosaic. The inoculation was made at the base of the stem. All leaves below the illustrated leaf gradually developed this type of chlorosis and died prematurely. Leaves above this one developed a mottling more nearly like that shown in Figures 5, B, and 7. The dark area near the apex was normal green as compared with leaves of the same stage of development on healthy plants. $\times \frac{2}{3}$



FIGURE 9.—Yellow mottling on a stem of Connecticut-Havana tobacco, induced by a concentrated virus of yellow mosaic. $\times 1$

The light-green type of mosaic from *Nicotiana glauca* is very similar in appearance, when transferred to tobacco, to the light-green mosaic which occurs on tobacco in the United States. It is

especially like the light-green mosaic which the writer has studied on tobacco (5). Differences in the properties of the viruses may exist, however.

The mild dark-green mosaic collected on *Nicotiana glauca* produces very little mottling on tobacco in the early stages of the disease. The first indication of disease consists of a bleaching of the veins. The leaves may become slightly stunted and more or less deformed. Sometimes a very faint green mottling may be noted at this period, but in most cases no mottling is evident. The later leaves show less

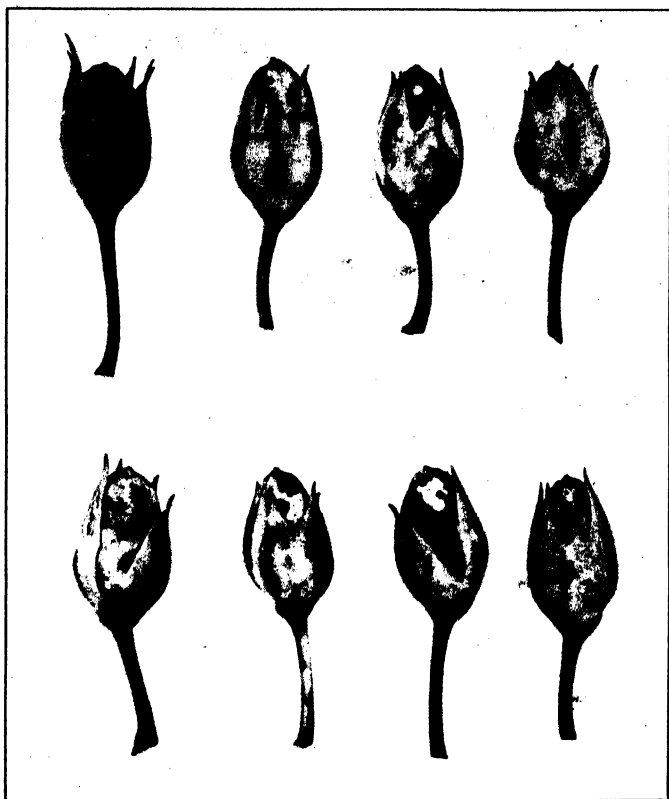


FIGURE 10.—Yellow mottling on the calyx and fruit of Connecticut-Havana tobacco, induced by a concentrated virus of yellow mosaic. Mosaic-free specimen at upper left. $\times 1$

deformity. They are normal green in color, and no mottling can be detected. From three to five such leaves may develop. These are followed by three to five leaves which show very small, faint light-green mottlings as shown in Figure 11. This condition becomes more intense on subsequent leaves until a very pronounced mottling occurs. This mottling is characterized by very large light and dark green areas (fig. 5, C), and sometimes more than half of a leaf is solid dark green and the remainder solid light green. The margins of the leaves frequently turn downward, causing an inverted spoon effect as shown in Figure 12. This stage may be followed by one in

which a few leaves develop very small, faint, light and dark green mottlings as in the case of the earlier stage. These observations are based on more than 50 plants which grew to maturity.

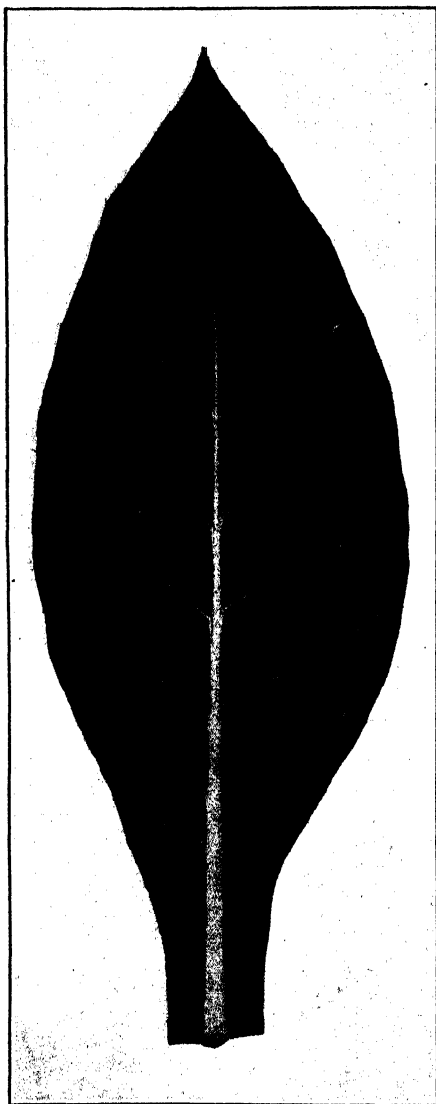


FIGURE 11.—Mild or faint mottling on Connecticut-Havana tobacco leaf, induced by a virus of mild dark-green mosaic collected on *Nicotiana glauca* in the Canary Islands. This mottling occurs in conjunction with the type illustrated in Figure 5, C. $\times \frac{3}{4}$

Thus far the mild dark-green mosaics have shown much less evidence of the yellow type than have the light-green forms, and at present it appears that the mild green types offer the best opportunity for obtaining a green mosaic free from yellow mosaic.

The writer has isolated a mild dark-green form from a slightly mixed infection occurring in *Nicotiana glauca* collected on Grand Canary, and this virus seems to be free from viruses causing the yellow and the light-green types of mosaic.

Inoculations have failed to transmit the mild dark-green mosaic of *Nicotiana glauca* to tomato plants grown from Canary Island seed as well as from American seed. The light-green and the yellow mosaics from *N. glauca* have been transmitted to tomatoes. Some of the tobacco mosaics of American origin have failed to produce symptoms in *N. glauca* collected on Teneriffe, but in some cases *N. glauca* has proved to be a carrier of these viruses after being inoculated. These same mosaics were transmitted by Johnson (2) to *N. glauca* from Italy, and symptoms were produced. From correspondence with Roy E. Clausen it is evident that all collections of *N. glauca* are not alike genetically. It appears, therefore, that the successful use of wild and domesticated hosts for differentiating viruses depends on the use of strains known to be homozygous for resistance or for susceptibility to a given mosaic. Obviously, workers in this field can not accurately

check one another's results until a stock of pure-line seeds is available.

When tobacco plants with mild dark-green mosaic were reinoculated with the virus of one of the writer's yellow mosaics, the

former type became obscured and the plants showed the typical symptoms of yellow mosaic. When plants with one of the writer's light-green mosaics were reinoculated with the same virus of yellow mosaic no change in the symptoms occurred. The plants continued to produce typical light-green mosaic after three reinoculations with the virus of yellow mosaic.

Leaf deformity is very frequently associated with mosaic on tobacco and *Nicotiana glauca*. Some of these deformities (fig. 12) occur with



FIGURE 12.—Extreme leaf deformations produced on Connecticut-Havana tobacco inoculated with a virus obtained from mosaic-diseased *Nicotiana glauca* collected at Gibraltar. One midrib had no lamina and another possessed only a small portion of a lamina. $\times \frac{1}{5}$

considerable regularity regardless of special environmental conditions, whereas others (fig. 13, A) occur only under certain limited conditions and do not continue throughout the life of the plant.

Some of the long narrow leaves shown in Figure 13, A, have distorted vascular elements. The veins are fused in many cases. Also the lamina is often distorted on the underside of the leaf forming liplike structures as shown in Figure 13, B. These leaf deformities are seemingly accentuated by reduced light, and they occur most frequently

in tobacco plants which are inoculated when very small. Woods (13) found that mosaic-affected tobacco produced deformed leaves after the plants had been cut back. The writer has obtained the same results with mosaic-affected tobacco plants and also with



FIGURE 13.—Mosaic on Connecticut-Havana tobacco: A, Deformed leaves of tobacco, produced by a light-green mosaic virus obtained from domestic tobacco. This plant was grown in shade during its early development after inoculation. This deformity does not continue as the plants develop, especially if they are given full sunlight. $\times \frac{1}{2}$. B, A portion of the underside of a deformed leaf showing the liplike structure in the lamina and the fusion of laterals with the midrib. $\times 2$

healthy plants. However, in both instances the leaf deformities did not continue throughout the life of the plants.

Other species of mosaic-free Solanaceae behave in the same manner. This phenomenon was observed very commonly in slashings of solanaceous perennials in the bush and the mountain jungles in Africa.

MOSAICS IN WEST AFRICA

The expedition reached Bathurst, capital of Gambia, early in January. Almost immediately a trip was made up the Gambia River to McCarthy's Island, which is located in the savanna type of vegetation.

In a garden located in the native village of Sankule, near Georgetown, mosaic was found on tabasco pepper and on eggplants. It was found again on cultivated eggplants in Monrovia, Liberia.

Colonial agricultural workers informed the writer that the rosette disease is very destructive on peanuts (*Arachis hypogaea* L.) in Gam-

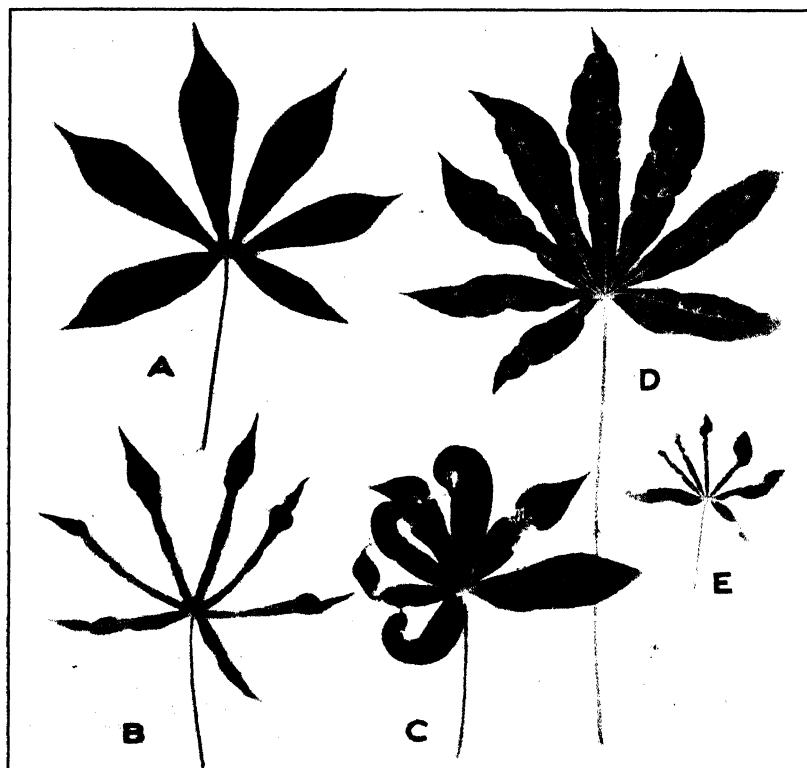


FIGURE 14.—Light-green mosaic on leaves of cassava (*Manihot* sp.): A, Healthy leaf; B-E, diseased leaves. Collected in Sierra Leone. $\times 1/4$

bia and in Sierra Leone. In some seasons this disease is the limiting factor in peanut production. The peanut crop had been harvested, and therefore it was not possible to observe rosette. This virus disease causes considerable damage in several regions in East, West, and South Africa (12). It has not yet been reported in the United States.

In all the colonies visited, from Liberia to French Cameroon, mosaic was prevalent on Cayenne, chili, and tabasco types of pepper (*Capsicum frutescens* L.) and on several types of cassava (*Manihot* sp.).

The mosaic on pepper is of the green type. It is identical in appearance with the mosaic commonly found on peppers in the United States, and it has been transmitted successfully by the writer to healthy chili pepper plants. Doubtless this pepper was taken to the West Coast in the days of the slave trade with the West Indies and South America.

The mosaic occurring on cassava is a yellowish green type and produces a considerable number of deformed leaves, as shown in Figure 14. Although this disease has not been studied, it has the appearance of an infectious mosaic, and it is considered as such by agricultural workers who are familiar with it. In many localities

the diseased plants were very much stunted, and the crop undoubtedly was reduced as a result.

The tuberous roots of the cassava supply much of the starchy food of the inhabitants of the Tropics of both hemispheres, and the tapioca of commerce is made from the starch derived from this plant. It seems apparent, therefore, that this disease should be studied intensively. Several slightly different kinds of cassava were observed in west Africa, and it is possible that some of these may offer an opportunity for making resistant selections.

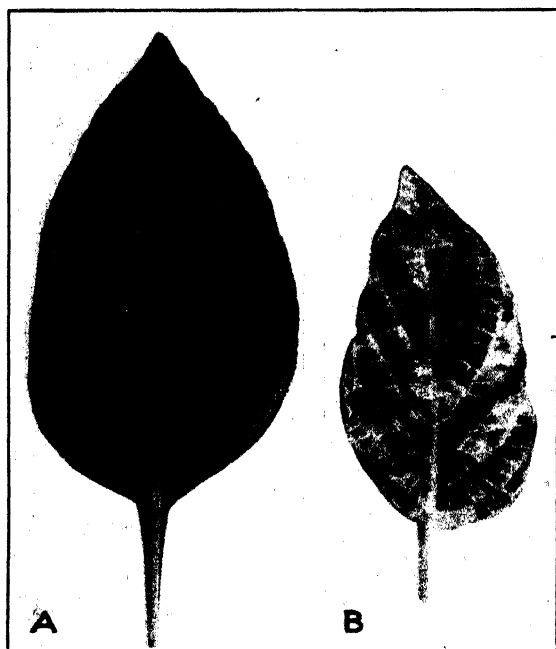


FIGURE 15.—Yellow mosaic on *Asystasia coromandeliana*: A, Healthy leaf; B, diseased leaf. Collected near Buea, British Cameroon. $\times 1$

The disease was noticeably less abundant on the lower elevations of Cameroon Mountain in the vicinity of Buea. A larger number of mosaic-free pepper plants also were found there than elsewhere. The district is somewhat cooler than the others visited, and it is possible that this has some bearing on the reduced amount of mosaic.

In Duala, French Cameroon, a yellow mosaic was found on *Asystasia coromandeliana* Nees (fig. 15), a weed which is rather abundant in that district. *Fleurya podocarpa* Wedd., a nettle, found on a jungle roadside near Buea, British Cameroon, had yellow mosaic.

The first evidence of mosaic on a cucurbit was found below Buea on Cameroon Mountain. A typical light-green type was found on *Physea barkeri* Cogn., a native wild species. (Fig. 16.) This mosaic resembled the one which occurs on the commercial cucumber

in the United States. The leaves on the mosaic-free plants were much larger than the diseased leaves.

Yellow mosaic (fig. 17) was found on a native cucurbit (*Momordica charantia* Linn.) at the Aburi Agricultural Experiment Station, Gold Coast, and on *Kedrostis foetidissima* Cogn. (fig. 18), a native cucurbit growing in a "bush island" (fig. 19) on the Winneba Plain in the Gold Coast. Plants of *Hibiscus calycinus* (fig. 20) growing 20 miles north of Accra, Gold Coast, also were found to have a yellow mosaic.

Many plants of the calabash gourd (*Lagenaria leucantha* Rusby), which were cultivated by the natives, were examined in all the colonies visited, but no suggestion of mosaic were found on this group of varieties.

The pepper and the eggplant were the only solanaceous plants that were found affected with mosaic in west Africa. Tomatoes cultivated near Buea, British Cameroon, were free from mosaic. In Gambia a few tomatoes of the cherry type, found in a native garden, had what appeared to be a fern-leaf type of disorder. Attempts to transmit this condition to tobacco failed.

Many wild species of Solanaceae were found in the Futa Jallon highlands, French Guinea, and on the lower elevations of Cameroon Mountain, but none showed mosaic.

Bananas and plantains were growing in many of the colonies visited. When possible, the plants were examined for indications of mosaic and bunchy top, but there was no evidence of these diseases.

Wild grasses were growing abundantly in the British and French Cameroons, in Fernando Po, and in parts of the Gold Coast. However, many observations failed to reveal any suggestion of mosaic on a grass.

Several types of sugar cane were found in all the colonies from the Gambia to the Cameroons. For the most part these plants were in native gardens. Some patches were found on the agricultural stations and farms, and one large planting was found near Cape Coast in the Gold Coast. Cane plants were carefully examined in all the localities, but no mosaic could be found. Infection experiments show that cane

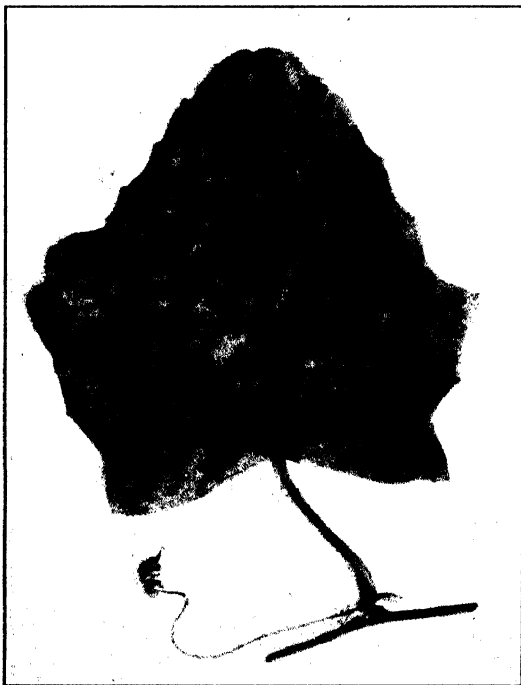


FIGURE 16.—Green mosaic on *Physedra barteri*. Collected near Buea, British Cameroon. $\times 2/3$

plants, from stalks collected in west Africa, are susceptible to the cane mosaic prevalent in Louisiana.

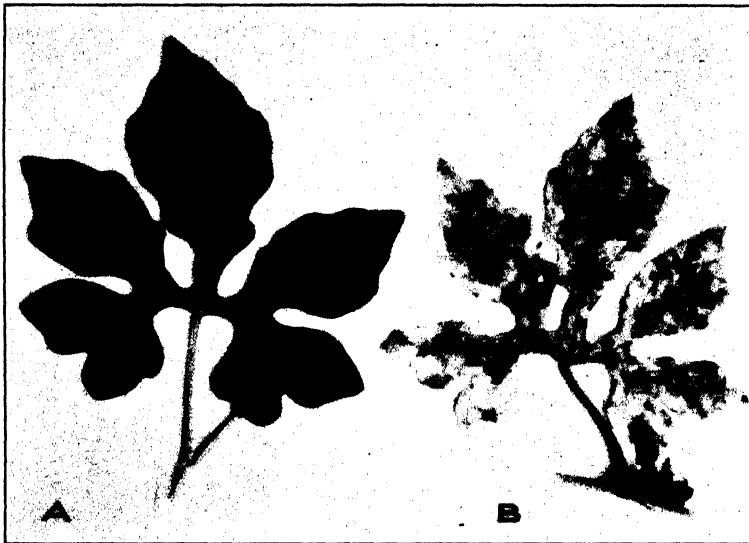


FIGURE 17.—Yellow mosaic on *Momordica charantia*: A, Healthy leaf; B, diseased leaf. Collected at Aburi, Gold Coast. $\times 1$

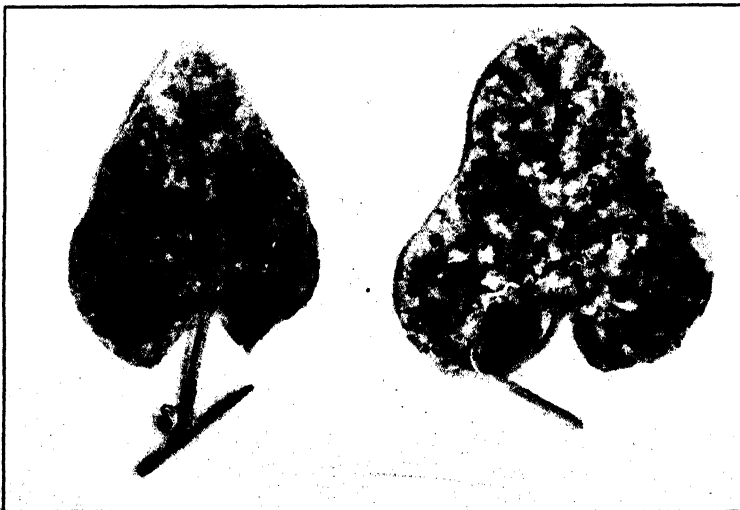


FIGURE 18.—Yellow mosaic on *Kedrostis foetidissima*. Collected in a "bush island" on the Winneba Plain, Gold Coast. $\times 1$

While in the Gold Coast the writer was informed by the Colonial mycologist that the streak disease occurs on maize in that colony. The disease could not be observed, as the crop had been harvested

some time previously. This is a destructive virus disease which occurs on the East Coast of Africa, in Egypt, and also in South Africa. It has not yet been reported from the Western Hemisphere.

From the literature and verbal descriptions obtained from workers who are familiar with streak, it appears to produce a stripe or streak which is similar in many particulars to the yellow mosaic associated with the green mosaic of wheat and other small grains. This yellow mosaic was mentioned and one type was illustrated by the writer several years ago (3). From the illustrations published by Storey (11), maize streak also resembles the Cuban maize stripe described by Stahl (10).

It is possible that Stahl considers streak and stripe distinct, because he could not transmit stripe to sugar cane, and because Storey failed



FIGURE 19.—Typical "bush islands" in the Winneba Plain, Gold Coast

to transmit streak with *Peregrinus maidis* Ashm., the vector for stripe. Illustrations of the two diseases are so similar that one is tempted to raise the question as to their possible identity in spite of vector differences. On the basis of published information it seems equally plausible that streak and stripe are identical and that *Peregrinus maidis* has biologic forms or races differing in their host preferences or in their ability to transmit a given virus. Certainly there is ample opportunity for such strain differences to manifest themselves in regions so widely separated as Africa and Cuba.

There is no information on the problem of biologic forms within species of insects serving as plant-disease vectors, and it is believed that this problem must be thoroughly explored in order that there may be a better understanding concerning vector relationships and possible differences among certain viruses.

EXPERIMENTS WITH A MOSAICLIKE DISEASE OF PEANUTS

When the writer was in Sierra Leone, E. Hargreaves, the colonial entomologist, called attention to a so-called mosaic on a few peanut plants he was carrying through the dry period in his compound at the agricultural experiment station at Njala, located more than 100 miles inland from Freetown. The writer took parts of diseased and healthy plants back to the yacht for inoculum and for obtaining photographic records. (Fig. 21.)



FIGURE 20—Yellow mosaic on leaf of *Hibiscus calycinus*. Collected near Accra, Gold Coast. $\times 1$

The tissue was placed in the ship's cool room until young peanut plants were available for inoculation. Plants were ready for inoculation in a few days, and at this time it was found that many aphids had been increasing on the diseased tissue. These were carefully removed to several of the young healthy plants. In from four to five days after this transfer was made the new leaves on the aphid-infested plants showed mottling identical with that on the original material. The control plants, which had no aphids, were free from mottling. As only one lot of aphids was available it was not possible to determine the effects from insects which had fed on healthy plants. However, it was considered that in case the mottling were an in-

fectious mosaic it would persist after the removal of the aphids. If it were the result of aphid attack, the plants would recover after the removal of the insects.

The aphids were removed from part of the mottled plants by means of a soapsuds spray. On these plants the new foliage was free from mottling, whereas the mottling continued on the new leaves of the plants which still harbored aphids. The mottlings developed again on the new foliage of recovered plants when aphids were allowed to feed on them again. The control plants which were kept free from aphids remained healthy.

The mottling differed somewhat from that of the ordinary types of green mosaic, and, from the behavior of the disease, it seems likely

that it is not a mosaic of the virus type. It seems to behave more nearly like some of the diseases of forage legumes which presumably are produced by the toxic products of certain species of leaf hoppers (8).

Unfortunately, the aphids were lost before they were identified, and further effort is necessary before identification is possible.

MOSAIC IN GIBRALTAR

On returning to Gibraltar late in March, the writer spent several days collecting. On one of the collecting trips a plant of *Nicotiana glauca* was found growing from a crevice in the side of the rock facing the Mediterranean Sea near Europa Point. This plant was just within reach, and on examination it was found to have a mild form of green mosaic. The virus from the dried leaves of this plant produced mosaic in tobacco plants one year after the collection was made. This mosaic has been studied only in a preliminary way. It appears to be very similar to the mild dark-green types collected

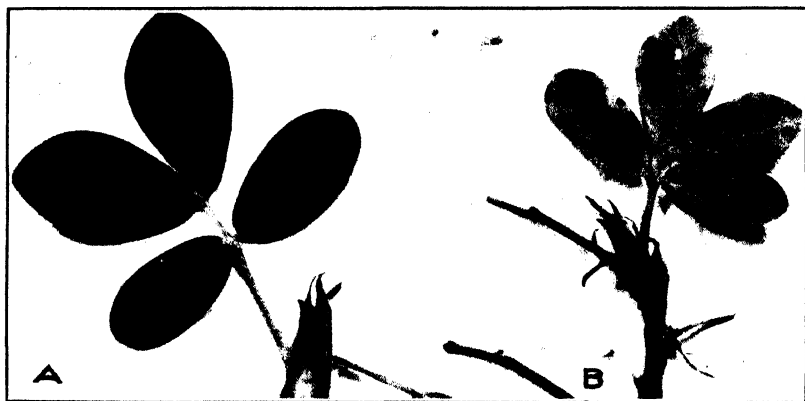


FIGURE 21.—Leaves of peanut (*Arachis hypogaea*): A, Healthy; B, mosaiclike mottling caused by aphids. Collected in Sierra Leone. $\times \frac{3}{10}$

in the Canary Islands. It frequently produces extreme leaf deformity, as shown in Figure 12.

DISCUSSION

The writer recognizes the importance of making inoculation tests before concluding definitely that a chlorotic condition is an infectious mosaic. It has not been possible to make inoculation tests with all of the collections, and it is possible that some of the mosaics described are not caused by viruses. However, in all the cases described, the symptoms were very typical for green or for yellow types of mosaic.

Mosaics of *Nicotiana glauca* obtained on Grand Canary, Tenerife, and Gibraltar are of considerable experimental interest.

The collections from the Canary Islands have been studied more thoroughly than the others, and it has been found that each mosaic-diseased specimen was affected by more than one type of mosaic. In all cases a yellow mosaic is associated to a greater or less extent with the green type, and in some cases it appears that two green mosaics and the yellow type are associated in the same plant.

Evidence obtained thus far indicates that the yellow mosaic is associated more generally with the light-green mosaics, and at the present time the mild dark-green types seem to offer an opportunity for obtaining a single virus of green mosaic.

It is of especial interest that each of the 17 viruses of light-green mosaic which have been tested on tobacco, and in some cases on tomato, contains virus of yellow mosaic. These viruses were obtained from seven different States in the United States and from Hawaii, England, and the Canary Islands.

If these associations of green and yellow mosaic, which appear to be rather general, are due to simple admixtures of two independent types or strains of virus, it would seem that there would be more evidence of a relatively pure yellow mosaic in fields of tobacco and tomatoes and in wild *Nicotiana glauca*. The writer has observed yellow mosaic most frequently in southern-grown tomatoes, but even in this case the number of plants has been exceedingly small in comparison with the large number in which green mosaic predominated. The fact that a yellow mosaic was not reported on field tobacco until recently by Johnson (2) is good evidence that this form is much less general than the green types.

A satisfactory interpretation of the yellow and green mosaic associations can not be given at this time because so few data are available. It is possible that these are a simple admixture of independent viruses comparable to a mixed infection of two or more species of fungi. However, it seems entirely possible that viruses may become altered locally in the plant, thus producing mutations, to use this term in its broadest meaning.

No attempt has been made to classify the viruses causing the mosaics described in this paper. This can not be done until studies now under way have been advanced. Any method of classification depends largely on the purity of the viruses under study. Johnson's work shows that differential hosts, heat, and chemical treatments assist greatly in separating types of viruses. However, there are instances in which these methods have not yet proved effective, as in the case of the association of the yellow and green mosaics described previously (4).

The writer's work shows that the studies on virus purification and classification should not be confined to young plants. In many cases plants must be carried to maturity in order to determine mixtures of viruses and to obtain a full knowledge of the symptom produced by a given pure virus.

It appears also that classification work should be based on host material which is homozygous for resistance or susceptibility to the mosaics being tested. Also, attention should be directed to the influence of toxic or other plant products on the behavior of a given virus after it has been passed through several species of hosts. The importance of homozygous host plants has been strikingly evident in the work on wheat mosaic. In the case of tobacco, the writer obtains seed from a single plant of known history and propagates it vegetatively in the greenhouse under conditions which prevent its receiving pollen from other plants.

It is not known how general the association of yellow and green types may be among the mosaics of other groups of plants. This association was observed in winter wheat (3) in 1920 near Granite

City, Ill., and it has been evident in experimental plot tests conducted since that time. Experiments show that yellow mosaic occurs more frequently in certain species of small grains and in certain varieties within a given species. Further study is required to determine whether the yellow and green mosaic association in the small grains is in any way analogous to the one occurring in the Solanaceae. Results obtained thus far seem to indicate that the symptoms of mosaic types in the small grains are influenced in part by genetic factors in the hosts.

Yellow mosaics are more devastating than the green types that have been observed, and it is fortunate that they are rare.

SUMMARY

In the Canary Islands green mosaics were found on *Solanum tuberosum*, *Nothoscordum fragrans*, *Capsicum frutescens* var. *grossum*, *Fuchsia magellanica gracilis*, *Psoralea bituminosa*, and *Nicotiana glauca*. Yellow mosaic was found on three plants of *N. glauca*.

Experimental studies have been carried out with Connecticut-Havana tobacco inoculated with viruses from the 10 mosaics collected on *Nicotiana glauca* in the Canary Islands. The results of these tests show that the green forms of mosaic are of two types, a light green and a mild dark green. The green forms that have been studied thoroughly were found to contain, to a greater or less degree, traces of a yellow mosaic virus which can be concentrated by successive spot isolations and subinoculations. The light-green mosaics develop more yellow mosaic spots than do the mild dark-green forms, and it appears that a strain of the latter has been isolated which is free from viruses of the yellow and the light-green mosaics.

In west Africa green mosaics were found on eggplant, pepper, *Manihot* sp., and *Physedra barteri*. Yellow mosaics were observed on *Fleurya podocarpa*, *Asystasia coromandeliana*, *Momordica charantia*, *Kedrostis foetidissima*, and *Hibiscus calycinus*.

The rosette disease occurs on peanuts in Gambia and Sierra Leone, and maize streak occurs in the Gold Coast.

In Sierra Leone a mosaiclike mottling occurs on peanuts. This is produced by aphids and seems not to be caused by a virus.

In Gibraltar mild dark-green mosaic was found on *Nicotiana glauca*.

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STUDIES OF FIRE BLIGHT OF APPLE IN WISCONSIN¹

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INTRODUCTION

Fire blight, caused by *Bacillus amylovorus* (Burr.) Trev., is one of the major diseases of pomaceous fruits. The destructiveness and widespread occurrence of this disease have contributed toward making it the subject of numerous investigations. Inasmuch as most of the voluminous literature which has resulted has been well reviewed by Jones (17)³, Stewart (28), Brooks (5), and others a general review of literature appears to be unwarranted. Such discussions of previous work as seem necessary appear in the body of the paper under appropriate headings.

Fire blight varies greatly both in severity of occurrence and in difficulty of control. In Wisconsin, epidemic outbreaks are frequent and destructive. Numerous attempts of the apple growers of this State to control the disease by the commonly recommended methods have been attended with but indifferent success. This variability in the severity of occurrence of fire blight and the difficulty of its control in epidemic years have led to further studies of the disease under local conditions. In these studies it seemed advisable to seek first a more adequate understanding of the course of disease development in relation to variability. Two correlated lines of study were undertaken: (1) Studies of the development and control of the disease in relation to the natural environment and (2) detailed studies under partly controlled conditions in the laboratory and greenhouse of certain phases of the life history of the causal organism in relation to pathogenesis.

DEVELOPMENT OF THE DISEASE IN RELATION TO THE NATURAL ENVIRONMENT⁴

SEASONAL DEVELOPMENT RECORDS

Blossom development of five of the more important apple varieties grown at Gays Mills, Wis., was followed from the time the cluster buds began to open until petal fall. From the data gathered the blooming periods of each of the varieties under observation were determined. These data are expressed graphically in Figures 1 to 3. Twig development was followed by tagging and measuring at frequent intervals 20 terminal twigs, representatively distributed, on

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² The writer wishes to express his indebtedness to Dr. G. W. Keltt, under whose direction this work was done, for suggesting the importance of the problem and for helpful suggestions and constructive criticisms during the progress of the investigation and the preparation of the manuscript. Grateful acknowledgments are also made to Prof. L. R. Jones and Dr. A. J. Riker for helpful advice, and to the stockholders and manager of the Kickapoo Development Company of Gays Mills, Wis., for their cordial cooperation in making the field studies possible.

³ Reference is made by number (italic) to "Literature cited," p. 620.

⁴ Studies of the development of the disease in relation to the natural environment were conducted in commercial orchards at Gays Mills, Wis. For three growing seasons (1926-1928) the writer lived in a small laboratory in the orchards, thereby gaining an excellent opportunity for daily observations and records.

each a resistant and on a susceptible variety. From the data obtained the average length of the current year's growth in inches was computed. (Figs. 1-3.)

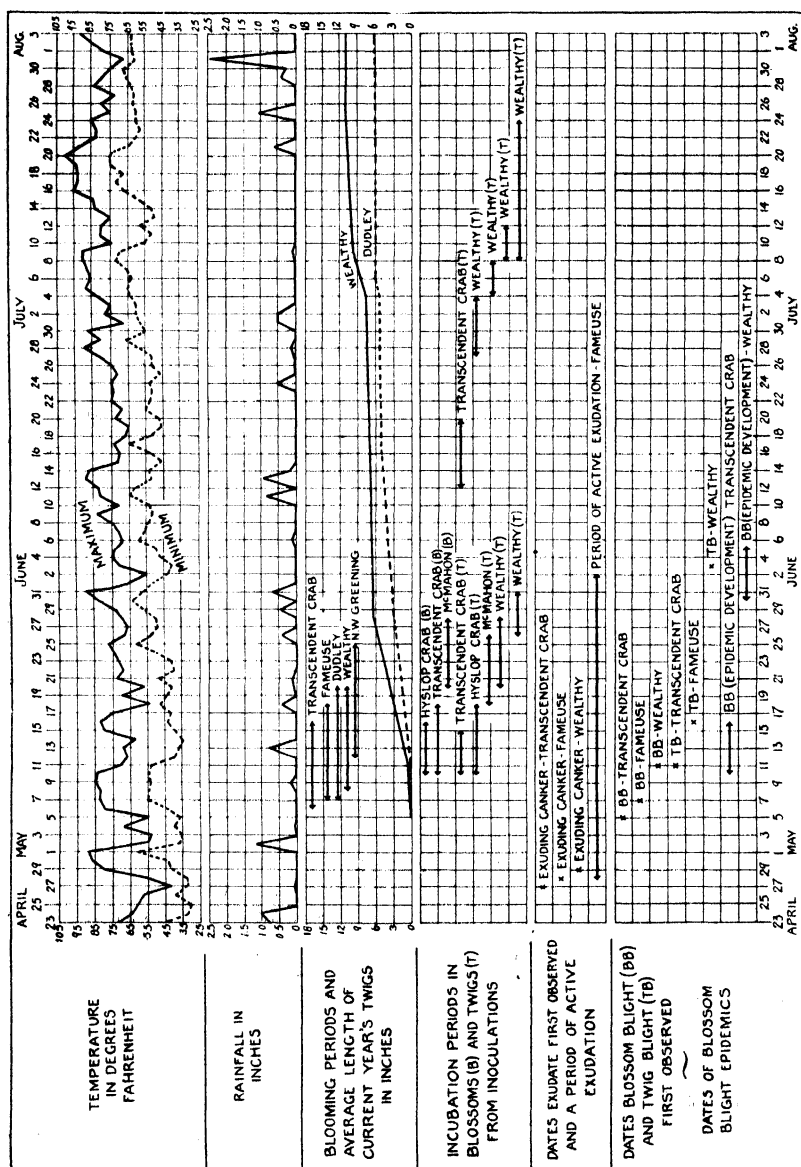


FIGURE 1.—Graphic summary of certain records pertaining to the epidemiology of fire blight on certain varieties of apple growing at Gay's Mills, Wis., and observed April 23 to August 3, 1926

A number of cankers which appeared likely to be active were tagged before any bacterial exudate was apparent. These cankers were examined daily, and the dates on which they started to exude and the subsequent dates of exudation were noted. Tags were placed

on 500 blossom clusters and 150 twigs representatively distributed on a number of Fameuse trees, and daily visits were made to each cluster and twig to determine whether blighting had taken place.

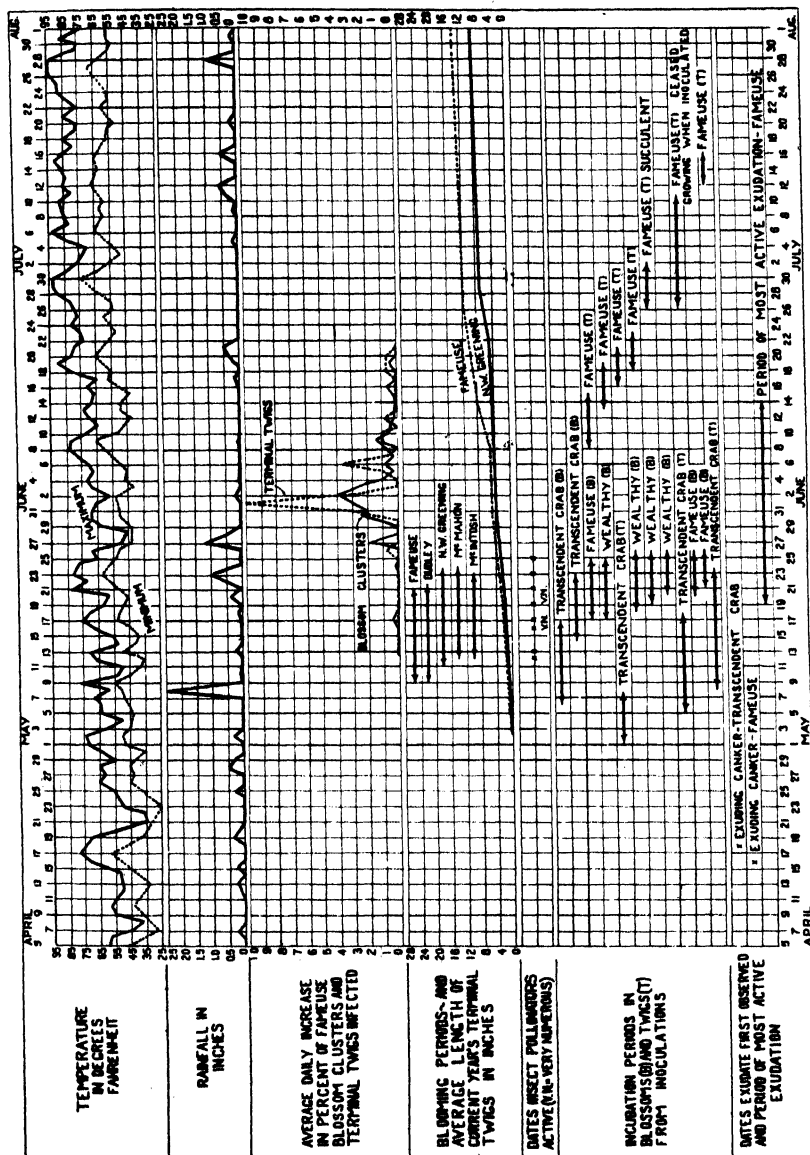


FIGURE 2.—Graphic summary of certain records pertaining to the epidemiology of fire blight on certain varieties of apple growing at Gays Mills, Wis., and observed April 5 to August 1, 1927

The results of these studies are presented graphically in Figures 1 to 3 in terms of the average daily increase in the percentage of terminal twigs and blossom clusters blighted.

Studies of the development of the disease under natural conditions were supplemented by artificial inoculation experiments. Unopened blossoms in the green-tip and early closed-cluster stages of development, and succulent twigs were inoculated from time to time by making wounds in the tissues with sterile needles and atomizing pure

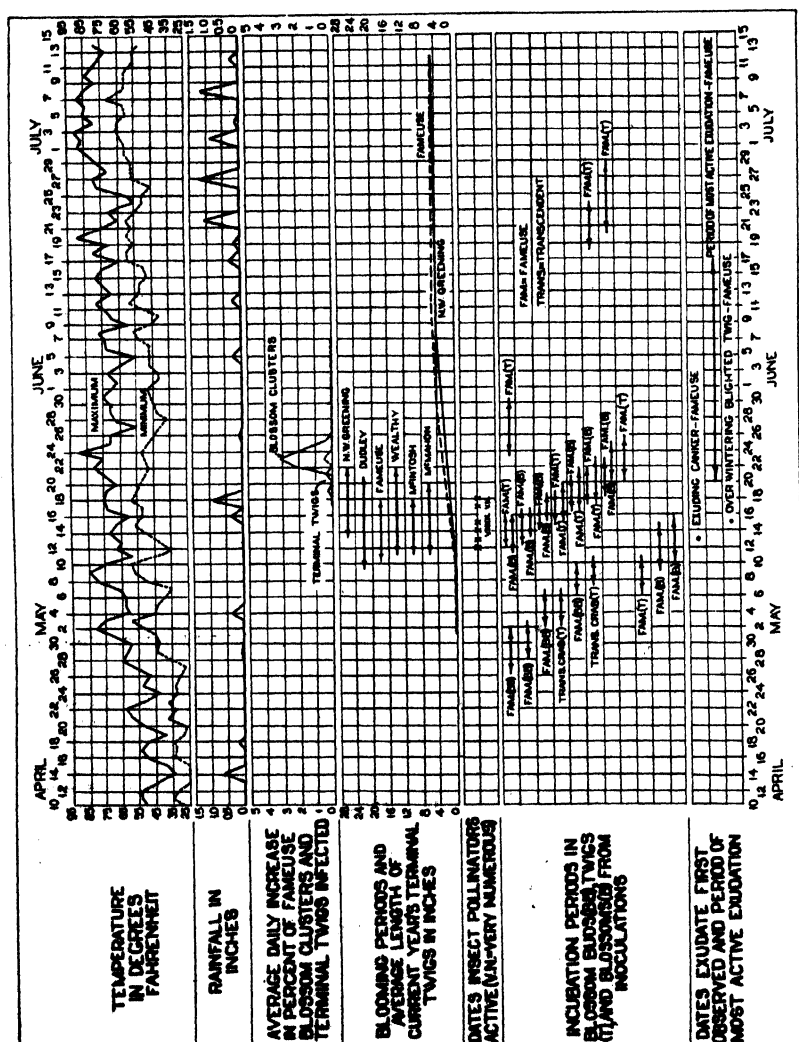


FIGURE 3.—Graphic summary of certain records pertaining to the epidemiology of fire blight on certain varieties of apple growing at Gays Mills, Wis., and observed April 10 to July 15, 1928

nutrient broth cultures of *Bacillus amylovorus* upon the wounded surfaces. Open flowers were inoculated at intervals starting from the time the blossoms first opened and continuing until several days after petal fall. These inoculations were made by spraying pure broth cultures of *B. amylovorus* into the receptacle cups with an atomizer. Before and after inoculation the unopened and open

blossoms were protected from natural infection by bagging. Daily visits were made to the inoculated blossoms and twigs and the incubation periods determined. The incubation periods as shown by these experiments appear in Figures 1 to 3. It is recognized that the data gathered in these studies on seasonal development are to be interpreted as approximately representative rather than as quantitatively exact.

METEOROLOGICAL RECORDS

The relative humidity and air temperature were recorded by means of a hygrothermograph which was housed in a standard instrument shelter set about 4 feet above the ground. The instrument was adjusted at frequent intervals by means of a sling psychrometer and a standardized thermometer.

Records of rainfall were taken by means of a standard rain and snow gauge.

DISCUSSION OF RESULTS

Yearly graphic summaries of disease development and meteorological data appear in Figures 1 to 3. While a more detailed analysis of these data will be presented later in relation to the various topics to which they are pertinent, it may be noted here that natural infection periods in both blossoms and twigs appear to be closely correlated with rain periods. Symptoms of the disease commonly became evident after a normal incubation period following rains, as was revealed by the inoculation experiments reported. The fact that twig blight begins to become manifest at approximately the same time as blossom blight is of striking significance in relation to the mode of dissemination of the natural inoculum. (See p. 588.)

FIELD STUDIES OF THE DEVELOPMENT OF EPIDEMICS

Fire-blight epidemics in Wisconsin are frequent and often very destructive. In some years the disease may be mild in its attack in one locality and severe in others. A knowledge of the chief factors concerned in the development of blight epidemics is obviously of great importance in relation to the control of this disease. It is sought in the following pages to trace the development of epidemics and to define critical periods in their occurrence and control.

MODES OF OVERWINTERING OF THE CAUSAL ORGANISM

HOLD-OVER CANKERS

The work of Waite (31), Whetzel (34), Jones (17), Brooks (5), and others has called attention to the fact that *Bacillus amylovorus* lives through the winter in the different hosts which it attacks largely in association with certain cankers which are known as "hold-overs." Only a comparatively few of the cankers formed in any one year, however, overwinter the causal organism, as most of the cankers are inactivated soon after their formation. Brooks (5) reports that 8 per cent of the cankers examined in Fameuse apple trees in 1925 were found to be hold-over sources of primary inoculum. The present writer found that 6 per cent of the cankers examined in Fameuse apple trees in 1926 overwintered the bacteria and in 1927, 7 per cent of those examined in this variety were hold-overs. In this work the extension

of the margins of the cankers in the spring and the production of exudate from the surface of the discolored areas have served to indicate those cankers which overwintered the causal organism. Only 3 per cent of the cankers examined in Fameuse trees in 1928 overwintered the bacteria. This scarcity of overwintering sources of primary inoculum may explain, in part, the sparse development of blight in 1928. While the percentage of cankers which overwinter the causal organism in cultivated apple trees is relatively small, it is usually sufficient, under Wisconsin conditions, to furnish a plentiful source of inoculum for primary infection in moderately or heavily infected orchards of the varieties which favor overwintering.

RELATION OF VARIETY OF APPLE

There is a marked difference in the amount of overwintering which commonly occurs in the various commercial apple varieties grown in Wisconsin. In the course of certain studies in 1924 and 1925 Brooks (5) observed this variation and states that "of 24 varieties of apple observed, the following were found to overwinter the organism and are listed in the order of their importance in this respect in the situations studied: Transcendent, Hyslop, Yellow Transparent, McMahon, Wealthy, Fameuse, Tolman Sweet, McIntosh, and Dudley." While the present writer has made no attempt to classify all the varieties grown in Wisconsin in respect to their importance in overwintering the fire-blight organism, marked differences in this regard have been noted during the course of these studies. In each year that this disease has been studied by the writer the Fameuse, Transcendent, Yellow Transparent, and Wealthy varieties were found to bear a much larger percentage of hold-over cankers than the McIntosh, Dudley, or Northwestern Greening. The Fameuse and Transcendent varieties were found to be particularly serious sources of primary inoculum as they contained the largest percentage of hold-over cankers in the varieties studied. (Table 1.) Such variations

TABLE 1.—*The per cent of cankers in certain apple varieties found to overwinter Bacillus amylovorus, Gays Mills, Wis., 1926-1928*

Year and variety	Cankers examined (number)	Cankers active	
		Number	Per cent
1926			
Fameuse	265	15	6
Wealthy	328	8	2
1927			
Fameuse	2,200	146	7
Wealthy	522	9	2
Dudley	375	1	0
McIntosh	13	0	0
1928			
Fameuse	1,483	38	3
Wealthy	310	2	1
Dudley	310	1	0

in the amount of overwintering which commonly occur in apple varieties may explain, in part at least, the failure of certain investigators to find overwintering sources of primary inoculum in certain localities. It is obviously difficult to find hold-over sources of primary inoculum in varieties which do not commonly overwinter the pathogene.

RELATION OF TYPE OF CANKER

An extensive examination of numerous cankers in 1926, 1927, and 1928 revealed an apparent relationship between the type of canker and its hold-over potentialities. For the most part those cankers which showed no line of delimitation about the periphery of the discolored areas in the fall were the ones most likely to carry the blight organism over the winter. In this type of canker the brownish, discolored portion merges into the apparently healthy tissues. (Fig. 4.) Those cankers which have a definite line of demarcation about the periphery of the cankerous area outside of which the bark shows no discoloration whatever are usually inactive and seldom carry the pathogene from one season to the next. In some instances, however, the bark surrounding the margin of certain delimited cankers is discolored. Such cankers often become active the following year.

POSITION OF THE BACTERIA IN HOLD-OVER CANKERS

On the basis of work done on the pear, Jones (17) states that the blight organisms "live over winter, not in the dead cankered tissue, but in the living bark immediately surrounding the cankered area." Brooks (5) concluded from a series of isolations from apple cankers that in the apple, as in the pear, the blight bacteria overwinter, not in the dead tissues of the canker, but in the apparently healthy tissues adjacent to the dead areas. Essentially the same results were obtained by the writer in studies on the position of the bacteria in overwintering apple cankers, although in one instance blight bacteria were isolated from the central portion of a hold-over canker. (Table 2.) Blight bacteria were recovered in a large percentage of the attempts from the tissues adjacent to the discolored margins of the cankers. All efforts to isolate the organism from apparently healthy tissues 2 inches beyond the discolored margin failed. From the work of Jones (17), Brooks (5), and the writer it seems fairly certain that the blight bacteria do not commonly overwinter in the brown discolored tissues of the canker, but that they are carried from one season to the next in the tissues immediately surrounding the periphery of the discolored margin of the canker. (Fig. 4.)



FIGURE 4.—A hold-over fire-blight canker on apple. There is no fissure about the boundary of the canker. The arrow points to the type of location, just outside the discolored area, from which the overwintering bacteria have commonly been isolated

TABLE 2.—Results of attempts to isolate *Bacillus amylovorus* from apple cankers^a

Series and dates of platings	Part of canker from which platings were made and results of trials								
	Discolored center			Living tissue 1 to 2 mm. beyond margin			Living tissue 2 inches beyond margin		
	Trials	Results		Trials	Results		Trials	Results	
		Posi- tive ^b	Nega- tive ^c		Posi- tive	Nega- tive		Posi- tive	Nega- tive
		Num- ber	Num- ber		Num- ber	Num- ber		Num- ber	Num- ber
Series 1, Apr. 11, 1926.....	8	1	7	8	7	1	8	0	8
Series 2, Feb. 28, 1927.....	25	0	25	24	3	21	25	0	25

^a Smooth-margined cankers on cut branches of Fameuse variety from Gays Mills.

^b Positive=*B. amylovorus* isolated.

^c Negative=*B. amylovorus* not isolated.

Few investigators have made histological studies to determine the mode and place of overwintering of the fire-blight bacteria. Nixon

(21), after a series of such studies, concluded that *Bacillus amylovorus* overwinters in an encysted condition within the cells. As further information on the mode and place of overwintering seemed desirable, a histological study was made of several smooth-margined cankers collected during the winter. Blocks of tissue, approximately 2 mm. square, from the center of the canker, from the living bark at the periphery of the canker, and from the apparently healthy bark 2 inches from the discolored margin, were fixed in formal acetic alcohol, embedded in paraffin, and sectioned.



FIGURE 5.—A hold-over blighted apple twig; the blight has extended down the twig and affected the two twigs of the current year's growth

The sections were stained with rose bengal (Conn, 7) and light green. No bacteria were found in the tissues taken from the central portion of the canker. However, in the tissues taken from the region just beyond the margin of the canker bacteria were found within the sieve tubes of the phloem. In many instances the bacteria were present in such numbers in the cells that the individual rods could be distinguished only with diffi-

culty. However, in no instance has a wall or sac been observed about the bacterial masses other than the cell wall of the sieve tube. The presence of true cysts, therefore, in overwintering blight cankers has not been verified in these experiments. In the apparently healthy tissues 2 inches from the margin of the canker no bacteria were found.

HOLD-OVER BLIGHTED TWIGS

Brooks (5) has shown that blighted apple twigs play an important rôle as sources of primary inoculum. His observations have, in the main, been confirmed by the writer. In some varieties a comparatively large number of blighted twigs (fig. 5) were found to overwinter the bacteria. In each of the three years that this disease was studied in the field the largest percentage of blighted twigs found overwintering the organism were on trees of the Fameuse variety. (Table 3.)

TABLE 3.—*The per cent of blighted apple twigs overwintering Bacillus amylovorus in certain apple varieties, Gays Mills, Wis., 1926-1928*

Year and variety	Blighted twigs examined (number)	Overwintering blighted twigs	
		Number	Per cent
1926			
Fameuse	1, 114	100	9
McMahon	406	6	1
Wealthy	500	1	0
1927			
Fameuse	1, 449	49	3
Wealthy	1, 701	2	0
McIntosh	342	1	0
Dudley	614	1	0
1928			
Fameuse	1, 581	15	1
Wealthy	500	2	0
Dudley	670	2	0
McIntosh	200	0	0

The size of blighted twigs which overwinter *Bacillus amylovorus* varies considerably. In 1926 the writer measured a number of twigs which had blighted the previous year and which were found to be overwintering the organism. A few of these measured only one-eighth inch in diameter at the point where the discolored tissue merged into the apparently healthy tissue. However, the great majority of overwintering twigs averaged approximately one-fourth inch in diameter. The size of the blighted twigs does not, therefore, limit overwintering.

RELATION OF THE POSITION OF THE HOLD-OVER SOURCES OF PRIMARY INOCULUM TO SUBSEQUENT DISEASE DEVELOPMENT

The position of hold-over cankers and overwintering blighted twigs in the trees seems to be of importance in determining the amount of current blossom and twig blight which develops. Observational evidence indicates that in those cases in which the overwintering sources of primary inoculum are found on limbs in the upper portion of the trees a comparatively large amount of both blossom and twig blight usually develops, if conditions are favorable, in the general region

below. On the other hand, in those trees in which the hold-over cankers occur in the crotch or on a limb near the ground line, little primary infection usually develops.

DISSEMINATION OF THE PRIMARY INOCULUM

The exact mode of dissemination of the inoculum which first establishes the fire-blight organism in the current year's growth is, obviously, of great potential importance in relation to control measures. Careful consideration was, therefore, given to the factors concerned in the spread of the primary inoculum.

RELATION OF INSECTS

The classic studies of Waite (30) clearly demonstrated that bees and other pollinating insects may carry the bacteria from blossom to blossom where they induce blighting. The mode of dissemination of the primary inoculum from hold-over sources to the blossoms and other susceptible parts has not been clearly demonstrated, however, either by observation or experiment. The prevailing idea has been that insects carry the inoculum from cankers where the organism overwinters to the susceptible parts of the current year's growth. Brooks (5) has suggested the possibility that some of the initial infection of the fruit spurs and twigs may be brought about by inoculation by aphids. He found them crawling up and down the large branches and also upon the trunks, and he states that "in their wanderings they traveled over exuding cankers and were observed many times to be caught in the bacterial exudate." Observations and experiments conducted by the writer at Gays Mills for three consecutive seasons, however, have failed to confirm the view that insects are important agents of dissemination of the primary inoculum under Wisconsin conditions. In support of this conclusion the following observations and experiments are cited:

(1) Although many hours were spent watching exuding cankers early in the spring in 1926, 1927, and 1928, pollinating or other insects have never been observed by the writer feeding upon or in contact with the exudate coming from the hold-over sources of primary inoculum.

(2) In each of the three years that this disease has been studied in the field there was a dearth of insects in the orchards in the spring and only a relatively few aphids and leaf hoppers were found until later in the season.

(3) Despite the scarcity of insects, numerous blight infections were found in the spring. The blight became apparent early and occurred in all the observed cases beneath hold-over sources of primary inoculum.

In the latter part of May, 1927, a few ants were observed passing over an exuding canker on their way to and from aphids, but extensive observations have failed to indicate that they are of importance in the initial spread of the disease. The rôle of insects in the spread of the primary inoculum appears to be less important, therefore, than has commonly been thought and needs to be reevaluated.

RELATION OF METEORIC WATER

Although Gossard and Walton (10) and Brooks (5) called attention to the spread of fire blight by meteoric water they did not recognize the importance of this agency as a disseminator of the primary

inoculum. Gossard and Walton (10, p. 107) state that “* * * raindrip does not become an agent of dissemination until primary centers of infection have been established, in nearly all cases, by insects.” The conditions encountered at Gays Mills, in the seasons of 1926 to 1928, inclusive, have offered a very favorable opportunity to follow the details of dissemination of the primary inoculum under natural conditions. In 1926 and 1927 young blossom clusters and leaf buds were found blighted well in advance of the opening of the blossoms and at a time when there was a striking dearth of insects which might have carried the bacteria from sources of primary inoculum. These diseased blossom and leaf buds were grouped beneath and in the vicinity of exuding cankers or overwintering blighted twigs so that conelike areas of infection resulted, with the sources of primary inoculum at the apices of these areas. The earliest infections of both open-blossom clusters and young shoots have also been observed to occur, almost without exception, below hold-over cankers or twigs in positions favorable for water-borne dissemination of the bacteria from these sources. The further fact was observed that the earliest infections of young shoots became evident at approximately the same time that blossom infections became manifest (figs. 2 and 3), and in many instances blighted blossoms and shoots occurred together in the same infection area.

Additional evidence of the importance of meteoric water in disseminating the primary inoculum was obtained from statistical studies conducted in the field in 1926 to 1928, inclusive. In each of these years the earliest blossom infections were found beneath exuding sources of primary inoculum. A close correlation was found to exist between the abundance of hold-over lesions in the trees and blossom infection. The writer's attention was first directed to this relationship in 1926 during the course of a survey of a group of Transcendents which were growing in the midst of a tract of McMahon apple trees. These Transcendents, of which there were only four, stood within 200 feet of one another, and each tree had approximately the same number of blossom clusters. In two of the trees (Table 4) there was considerable blossom blight, whereas in the other two only an occasional blighted blossom cluster could be found. The diseased clusters in trees 1 and 2 were found only in certain portions of the trees and were grouped in conelike areas of infection. At the apices of these blighted areas hold-over cankers were found.

TABLE 4.—*The relation between the abundance of sources of primary inoculum and the development of blossom blight on Transcendent apple, Gays Mills, Wis., 1926*

Tree No.	Sources of primary inoculum observed		Blossom clusters blighted
	Cankers	Twigs	
	Number	Number	Per cent
1	3	0	23
2	1	0	15
3	0	0	0
4	0	0	0

Further evidence of the importance of meteoric water as an agent of dissemination of the primary inoculum was obtained from studies of the relation of the abundance of overwintering sources of primary inoculum to blossom-blight development in Fameuse trees in certain of the control plots at Gays Mills in 1926, 1927, and 1928. In these plots an attempt was made to remove all cankers and blighted twigs while the trees were dormant. In several plots, however, a number of hold-over cankers and overwintering blighted twigs were overlooked during the excision process. Blossom-blight counts made in these plots revealed a close correlation between the abundance of overwintering sources of primary inoculum in the trees and the amount of blossom blight. (Table 5.) In some instances where the hold-over lesions were located on the lower limbs or in the crotches of trees in situations unfavorable for water-borne dissemination of the inoculum this correlation was not so apparent.

TABLE 5.—*The relation of local sources of primary inoculum to the development of blossom blight on Fameuse apple in plots where excision had been practiced,^a Gays Mills, Wis., 1926-1928*

Year and tree No.	Blossom clusters		Hold overs ^b		Year and tree No.	Blossom clusters		Hold overs	
	Examined	Blighted	Cankers	Twigs		Examined	Blighted	Cankers	Twigs
	Number	Per cent	Number	Number		Number	Per cent	Number	Number
1926									
1	121	1	0	0	41	151	14	0	1
2	45	0	0	0	42	55	0	0	0
3	52	4	0	0	43	134	33	2	0
4	113	33	1	0	44	129	3	0	0
5	108	44	2	1	45	100	0	0	0
6	100	0	0	0	46	100	0	0	0
7	103	3	0	0	47	100	0	0	0
8	165	48	2	1	48	117	14	1	0
9	129	22	0	1	49	101	1	0	0
10	112	11	0	1	50	63	5	0	0
11	50	0	0	0	51	141	43	3	0
12	50	0	0	0	52	100	0	0	0
13	50	0	0	0	53	107	6	0	1
14	100	0	0	0	54	60	0	0	0
15	50	0	0	0	55	116	1	0	0
16	100	0	0	0	56	70	0	0	0
17	101	1	0	0	57	120	5	0	0
18	77	0	0	0					
19	52	4	0	0	1927				
20	50	0	0	0	1	60	0	0	0
21	86	37	1	0	2	75	0	0	0
22	50	0	0	0	3	51	2	1	0
23	111	10	3	0	4	50	0	0	0
24	70	0	0	0	5	111	1	0	0
25	50	0	0	0	6	85	0	0	0
26	40	0	0	0	7	191	1	0	0
27	75	33	1	1	8	78	4	0	0
28	142	44	3	0	9	127	2	0	2
29	100	0	0	0	10	211	3	0	1
30	104	4	0	0	11	111	1	0	0
31	95	7	0	0	12	278	6	0	0
32	83	19	1	3	13	225	11	0	3
33	96	11	1	0	14	144	6	1	0
34	75	0	0	0	15	141	1	0	0
35	65	0	0	0	16	69	6	0	0
36	127	8	0	0	17	140	0	0	0
37	56	11	1	0	18	80	0	0	0
38	90	55	4	0	19	140	0	0	0
39	51	39	2	0	20	200	0	0	0
40	101	1	0	0	21	85	6	0	1

^a Blossom-blight counts were made usually the third week after petal fall. In determining the percentage of blossom blight, flower clusters in all parts of the tree were examined so as to obtain representative counts.

^b Sources of primary inoculum observed. For the most part, the blight infections observed were grouped beneath and in the vicinity of these hold-overs.

TABLE 5.—*The relation of local sources of primary inoculum to the development of blossom blight on Fameuse apple in plots where excision had been practiced, Gays Mills, Wis., 1926-1928—Continued*

Year and tree No.	Blossom clusters		Hold overs		Year and tree No.	Blossom clusters		Hold overs	
	Examined	Blighted	Cankers	Twigs		Examined	Blighted	Cankers	Twigs
	Number	Per cent	Number	Number		Number	Per cent	Number	Number
22	105	0	0	0	30	155	6	0	1
23	100	0	0	0	31	75	0	0	0
24	205	44	4	3	32	80	0	0	0
25	135	0	0	0	33	40	2	0	0
26	152	8	0	1	34	150	0	0	0
27	278	1	0	0	35	60	0	0	0
28	112	2	0	0	36	160	0	0	0
29	199	2	0	0	37	40	0	0	0
30	214	2	0	1	38	115	0	0	0
31	76	2	0	0	39	160	0	0	0
32	130	0	0	0	40	60	0	0	0
33	71	1	0	0	41	200	1	0	0
					42	155	0	0	0
					43	85	0	0	0
1928					44	200	0	0	0
1	110	0	0	0	45	100	0	0	0
2	120	0	0	0	46	140	2	0	0
3	40	0	0	0	47	100	8	0	1
4	150	0	0	0	48	100	2	0	d 2
5	200	0	0	0	49	100	0	0	0
6	100	0	0	0	50	100	0	0	0
7	200	0	0	0	51	100	0	0	0
8	120	0	0	0	52	90	1	0	c 2
9	130	0	0	0	53	100	0	0	0
10	130	0	0	0	54	100	0	0	0
11	100	0	0	0	55	100	0	0	0
12	100	0	0	0	56	100	17	2	2
13	110	0	0	0	57	100	0	0	0
14	90	0	0	0	58	100	0	0	0
15	160	11	1	0	59	100	0	0	0
16	140	1	0	0	60	110	1	0	0
17	130	0	0	0	61	100	2	0	0
18	75	1	0	0	62	100	0	0	0
19	175	0	0	0	63	100	0	0	0
20	140	0	0	0	64	120	0	0	0
21	75	0	0	0	65	90	9	0	c 3
22	90	0	0	0	66	45	0	0	0
23	100	0	0	0	67	45	11	0	1
24	225	0	0	0	68	50	0	0	0
25	60	0	0	0	69	85	0	0	0
26	130	0	0	0	70	100	0	0	0
27	140	0	0	0	71	85	0	0	0
28	60	0	0	0					
29	115	0	0	0					

^c There were comparatively few blossom clusters underneath the exuding sources of primary inoculum in these trees.

^d The overwintering blighted twigs in this case were located in the lower portion of the tree. The fact that these sources of primary inoculum were unfavorably situated for water-borne dissemination of the bacteria probably accounts for the small amount of blight found in the trees.

In 1928 large numbers of blight-infection areas were found in late May and early June beneath hold-over lesions. Those trees which contained the largest number of overwintering sources of primary inoculum favorably situated for the water-borne dissemination of the bacteria were commonly found to be the most severely blighted.

From the evidence that has been presented it appears that, under Wisconsin conditions, meteoric water is the most important agency concerned in disseminating the bacteria from hold-over sources of primary inoculum to the current year's growth.

DISSEMINATION OF THE SECONDARY INOCULUM

Following the work of Waite (30), Jones (17), Stewart (28), Burrill (6), and others it became generally accepted that the secondary inoculum is disseminated almost entirely by insects. It was thought that blossom blight was spread chiefly by pollinating insects and that twig blight was disseminated largely by such insects as aphids, leafhoppers, tarnished plant bugs, and others. An important modification of these earlier conceptions was necessitated by the valuable contributions of Gossard and Walton (10). From their studies they concluded that from 50 to 90 per cent of the blossom blight which they observed was caused by rain-borne inoculum. In somewhat similar experiments, which appear to have been conducted independently and near the same time, Stevens, Ruth, and Spooner (27) showed that blossom clusters and young twigs which were carefully protected from visitation by insects were blighted approximately as much as similar unprotected parts. Access of air and of meteoric water was not precluded. The writers conclude that the disease must have been transmitted by some agency other than insects, and state: "The only tenable hypothesis is that wind was the chief agent of transmission." The present writer's studies furnish additional evidence that meteoric water is an important agent in the dissemination of the secondary inoculum for fire blight and that the rôle of insects in the spread of the disease is less important than was earlier believed. In support of this view the following observations and experiments are pertinent.

(1) In each of the three years that this disease was studied under natural conditions, trees varied greatly in the severity of blossom blight, and blighted clusters were often found grouped in conelike areas of infection. In some instances practically every blossom cluster in one part of a tree was infected, whereas in other portions of the same tree only scattered clusters were diseased. No hold-over sources of primary inoculum could be detected in certain trees showing such conelike areas of infection. Instead of hold-over lesions, early blighted blossom clusters were found to be the source of inoculum. If all blossom infections are the result of inoculations by insect carriers of fire blight a more uniform distribution of blight infections in the trees should be expected with regard both to the trees infected and the distribution of blighted blossoms on individual trees.

Many other observations pointed to a more localized spread of blight than would be expected if pollinating insects were the chief disseminators of the secondary inoculum. In 1927, for example, a considerable local development of blossom blight was found in one corner of a McMahon tract, the remainder of which showed a very sparse development of blossom blight. The trees which were heavily infected were grouped about a badly blighted Transcendent in which were found hold-over lesions. No hold-overs were observed in the badly blighted McMahon trees and only rarely occasional cases of overwintering of the organism were found on the McMahons in this block. The amount of blossom blight diminished rapidly as the distance from the Transcendent increased.

A similar example on a larger scale was observed in the same year. A large block of Fameuse which contained numerous hold-over lesions and was heavily infected with blossom blight adjoined a block of Dudley in which only occasional hold-over lesions were found. The

blooming periods of the two varieties were approximately identical. (Fig. 2.) In the Dudley trees immediately adjacent to the Fameuse block abundant blossom blight developed. The amount of blight, however, diminished rapidly as the distance from the Fameuse block increased, most of the disease being confined to five or six rows nearest the Fameuse. The more remote parts of the Dudley block, including some 10 acres, remained practically free from blossom blight.

(2) Attention was further directed to the important rôle played by meteoric water in the secondary spread of fire blight in experiments which were conducted in 1928 at Gays Mills. Two apple trees, a Fameuse and a Wealthy, containing numerous blossom buds in the early closed-cluster stage of development, were sprayed with Derrisol (1-800) to kill any aphids which might have been present. These trees were then inclosed in cheesecloth insect-proof cages with the aim of excluding all pollinating and other insects.



FIGURE 6.—Bacterial exudate coming from the pedicel of a pear flower inoculated by atomizing a pure culture of *Bacillus amyloporus* into the receptacle cup. Photograph taken three days after inoculation

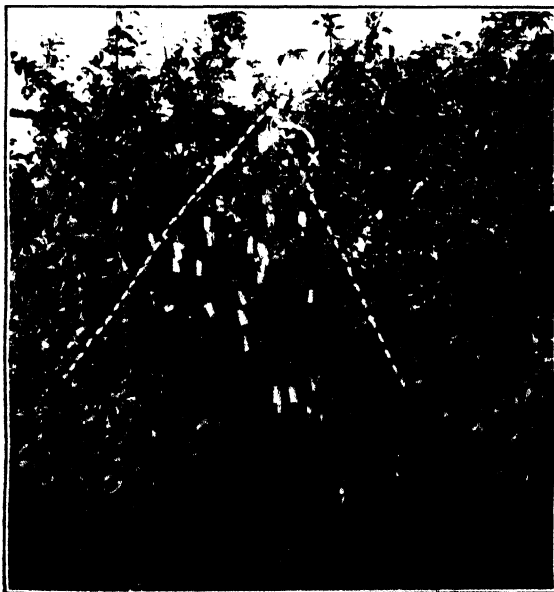


FIGURE 7.—A blighted Fameuse apple tree. Before the blossoms opened this tree was inclosed in a cheesecloth cage to keep out pollinating insects, and two blossoms clusters at X were inoculated. Abundant secondary infection of blossom clusters developed in a cone-shaped area. Each infected blossom has a white tag attached

developed with the inoculated flower clusters at the apices of these diseased areas. (Fig. 7.) Examinations failed to reveal the presence of any

Several early blooming flower clusters in the top of each of the trees were inoculated on May 10 with a pure suspension of *Bacillus amyloporus* in sterile distilled water by carefully inserting the inoculum into the receptacle cups. Bacterial exudate was found on May 15 coming from the pedicels of the inoculated flowers. Three-tenths inch of rain fell the following day (May 16), and on May 18 there was a precipitation of nine-eighths inch. On May 23, five days after the last of these two rains, a number of flower clusters were found beneath the inoculated ones showing fire-light symptoms. Conelike areas of infection

insects. Meteoric water, therefore, appears to have been the only agent which could possibly have disseminated the inoculum.

(3) In 1926 at Gays Mills aphids were scarce in the spring but numerous in midsummer. In 1927 both leaf hoppers and aphids were relatively numerous from the middle of June throughout the growing season. In spite of their relative abundance, however, twig blight was not at all serious either in 1926 or 1927, except in a relatively small number of trees scattered here and there throughout the orchard. In virtually all of the trees which were infected either blighted blossom clusters or overwintering sources of primary inoculum were discernible in some part of the trees. This relationship was very apparent in certain trees in some of the plots at Gays Mills in which an attempt was made to remove all overwintering sources of inoculum in the fall of 1925. The following spring several trees in these plots were found to contain an abundance of both infected blossoms and shoots. The disease development observed in these trees was found upon examination to be traceable to overwintering cankers which had been overlooked during the excision process the preceding fall. The adjoining trees, while heavily infested with leaf hoppers, contained only a scattered infection here and there. If the widespread dissemination of twig blight is to be attributed largely to sucking insects, theoretically we should expect a cross transfer of the inoculum from heavily infected trees to adjacent ones. This is not in accord with the writer's observations, which extend over a 3-year period. It would seem, therefore, that a local source of inoculum either in the form of hold-over cankers, overwintering blighted twigs, or diseased flower clusters or shoots must be present in the trees before twig blight becomes serious. (Table 6.)

TABLE 6.—*The relation of the number of blighted blossom clusters in Fameuse apple trees to twig-blight development, Gays Mills, Wis., 1927*

Tree No.	Blighted blossoms	Blighted twigs	Tree No.	Blighted blossoms	Blighted twigs
	Number	Number		Number	Number
1.....	9	11	22.....	15	39
2.....	13	6	23.....	1	2
3.....	0	1	24.....	0	1
4.....	3	3	25.....	0	0
5.....	1	2	26.....	0	2
6.....	0	0	27.....	0	0
7.....	0	1	28.....	0	0
8.....	2	0	29.....	0	0
9.....	0	0	30.....	0	0
10.....	0	0	31.....	0	0
11.....	3	1	32.....	14	10
12.....	9	5	33.....	2	4
13.....	2	9	34.....	120	29
14.....	12	11	35.....	4	24
15.....	0	0	36.....	0	0
16.....	0	0	37.....	0	0
17.....	0	0	38.....	0	1
18.....	7	8	39.....	0	1
19.....	2	6	40.....	0	1
20.....	14	56	41.....	3	0
21.....	51	6	42.....	0	1

While the work of Waite (30), Gossard and Walton (10), and others has shown that pollinating insects may spread fire blight, there are practically no data available which would clearly indicate the

extent to which they are concerned in the dissemination of the disease. Does a bee disseminate the disease extensively after visiting a diseased flower, or does it cease to be a carrier of blight after visiting a comparatively few flowers? This is a very important point in relation to the epidemiology of fire blight and deserves critical consideration. In 1928 the writer made a preliminary attempt to gain evidence on this problem, but due to the limited amount of material available only a beginning was made toward its solution. In experiments conducted in the greenhouse the proboscis of a bee was dissected out and tied to the end of a small stick of wood. The tip of the distal end was then dipped in melted paraffin in such a manner as to seal the opening to its interior. The proboscis and its carrier were sterilized in 95 per cent alcohol and washed through several changes of sterile water. The distal portion of the proboscis was then dipped into the nectarial fluid of a pear flower which had been inoculated with *Bacillus amylovorus* three days earlier. The proboscis was next dipped consecutively into the nectar in the receptacle cups of 15 healthy pear flowers. Every one of the flowers thus inoculated blighted in a very characteristic manner. This would seem to suggest that pollinating insects may still be potential agents of dissemination even after having visited 15 flowers following contamination.

THE MODE OF ENTRY OF *BACILLUS AMYLOVORUS*

INTO YOUNG SHOOTS AND UNOPENED FLOWERS

It has long been accepted that access of *Bacillus amylovorus* to the host tissues is accomplished in two ways: (1) Through the open blossoms, presumably through the uninjured nectaries in the receptacle cup (Waite (30, 31)) and (2) through wounds of many kinds (Jones (17), Stewart (28), Burrill (6), Heald (13), and others). On the basis of observations, Heald (13) expressed the opinion that the bacteria may gain entrance to the leaves also through water pores and stomata but stated that it remained for further investigation definitely to substantiate this view. He states (14, p. 315):

The writer studied fire blight in Washington in the summer of 1915 and found leaf invasions common in pear, apple, and quince, and later produced artificial infections through the leaf margins by the use of pure broth or bouillon cultures. Leaf infections occurred through marginal breaks, insect punctures or through perfectly sound leaves.

Brooks (5) reported negative results from numerous inoculation experiments with unwounded apple leaves in the field and greenhouse. Most of this work was done, however, after the twigs had made considerable growth.

The occurrence at Gays Mills of blighted cluster buds and infected young shoots early in the season in the absence of insects and in association with hold-over sources of primary inoculum suggested to the present writer the hypothesis that the bacteria are able to infect young shoots and unopened blossoms without the intervention of wounds. Attention was, therefore, directed to the manner in which the bacteria gain access to the tissues of the host. Working with potted apple and pear trees grown in the greenhouse under conditions designed to prevent injury from insects and other agencies, the writer

induced infection of young shoots and unopened blossom buds of apple and pear at will by spraying them with a pure suspension of *Bacillus amylovorus* in sterile distilled water and placing them for varying periods in a moist chamber⁵ at suitable temperatures. After inoculation the plants were incubated in a well-illuminated chamber⁶ which was maintained at a temperature of 28° C. and 70 per cent relative humidity. Two types of disease development commonly followed. In those varieties which have a thick mat of hairs on the dorsal surface of the young leaves (as Wealthy and Fameuse) the first symptoms of the disease commonly appeared along the margin of the young leaves from four to six days after inoculation. From the margin the disease spread down the midrib if the lesions were terminal,



FIGURE 8.—Representative twigs grown under conditions designed to prevent injury, inoculated by atomizing with a pure suspension of *Bacillus amylovorus* in sterile distilled water, and demonstrating the entry of the fire-blight bacteria through stomata. Twigs on the tree at the left (A) are controls

or the veinlets if lateral, resulting in lesions which were more or less triangular in outline. The petioles of the infected leaves were then invaded, followed by infection of the main axis of the shoot. In a comparatively short time a decided wilting and typical blighting of the young shoots developed. (Fig. 8.) Bacterial exudate was found on the invaded areas in many instances. *B. amylovorus* was successfully recovered from the invaded areas in all of the numerous isolations attempted. In those varieties which have comparatively few hairs on the undersurface of the young leaves (as Transcendent or Whitney)

⁵ Through the kindness of Dr. G. W. Keitt, an infection chamber (Keitt and Jones (18)) was made available for these studies. Slight alterations were made in the method of maintaining the humidity. The desired temperature and humidity in the chamber were maintained in part by 250-watt luminous radiator unit covered with moist absorbent cotton. Moistened pieces of cheesecloth suspended from troughs of water around the walls of the chamber replaced the cloth inner chamber used by Keitt and Jones and aided in keeping the desired temperature and humidity. Hot or cold water from supplies kept at suitable constant temperatures were mixed through metal valves to give the desired temperature to the water in the troughs.

⁶ The writer is indebted to Dr. J. G. Dickson for his kindness in permitting the use of one of his temperature and humidity chambers for these studies.

a leaf spotting (fig. 9) often occurred, particularly, if a premoist treatment had been given the young shoots before inoculation. These lesions often coalesced and the disease invaded the midrib, petiole, and main axis of the shoot in a manner comparable to that outlined above. These two types of development following inoculation of young shoots were associated, in part at least, with the wettability of the dorsal surfaces of the young leaves. The older leaves seemed to be more or less resistant to the invasion of the bacteria, for only a very few became infected until the main axis of the shoot was invaded. In those that were infected the blight lesions were localized at the margins and failed to spread to any considerable extent. The factors which are responsible for the apparent resistance of the older leaves have not been fully analyzed.

Blossom buds inoculated and treated in the same manner as the young shoots showed symptoms of fire blight within three to six days. The tips of the exposed sepals and the apices of leaves surrounding the blossom buds turned brown (fig. 10), and under humid conditions a bacterial exudate was found coming from the discolored parts. In a number of instances isolations were made from the invaded areas with uniformly successful results. Four to six

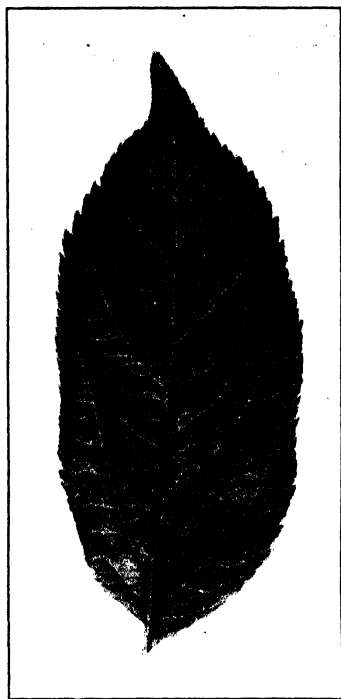


FIGURE 9.—Leaf from a young shoot of Whitney (crab) apple inoculated by atomizing with a pure suspension of *Bacillus amylovorus* in sterile distilled water; a leaf-spot symptom was commonly observed on this variety in greenhouse experiments during the earlier stages of disease development.



FIGURE 10.—Cluster of infected unopened apple blossoms. These buds were inoculated by atomizing with a pure suspension of *Bacillus amylovorus* in sterile distilled water. The apical parts of the sepals (a) and of one leaf (b) are diseased.

days after the appearance of the first symptoms of the disease, the tissues of many of the infected buds were all brown and dead, and evidence of the disease, in many instances, was found also in the 1-year-old wood. As is shown in Table 7, in those cases where the buds received a preliminary moist treatment before inoculation there was a larger percentage of infections than when no premoist treatment was given. However, in view of the positive results obtained without the administration of a preliminary moist treatment it seems that such treatments are not absolutely essential for infection.

TABLE 7.—Results of inoculation experiments with *Bacillus amylovorus* on unopened apple-blossom buds and young shoots grown under conditions designed to prevent injury from insects and other agencies, Madison, Wis., 1928

Parts inoculated and number of experiment	Period in moist chamber		Results				
			Inoculations			Controls	
	Before inoculation	After inoculation	Healthy	Diseased	Organism reisolated	Healthy	Diseased
	Hours	Hours	Number	Number		Number	Number
Twigs: ^a		48	1	2	+	4	0
1.....	{	96	1	1	+	5	0
		168	0	7	+	2	0
		72	3	1	+	3	0
2.....	{	96	3	1	+	4	0
		24	2	7	+	6	0
		46	0	4	+	4	6
3.....		40	2	9	+	2	0
4.....		26	1	4	+	6	0
5.....	{	24	4	9	+	8	0
		22	4	6	+	11	0
		24	0	9	+	3	0
6.....		19	0	2	+	1	0
Blossom buds: ^c							
1.....	{	24	0	1	+	2	0
		41	0	2	+	3	0
		22	0	3	+		
2.....	{	23	0	7	+	5	0
		22	5	1	+		
		19	1	4	+	5	0
3.....	{	22	0	1	+	1	0
		28	0	3	+	3	0
		16	0	2	+	2	0
4.....		23	0	2	+	3	0
5.....		20	1	2	+	1	0
6.....		24	0	6	+		
7.....		27	0	2			
8.....		6					

^a Young succulent shoots, 2 to 7 inches long.

^b + = *B. amylovorus* reisolated.

^c Apple blossom buds in the green tip and early closed cluster stage of development.

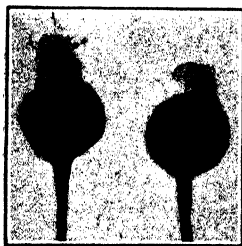


FIGURE 11.—Fire-blight lesions on young apple fruits inoculated 10 days after petal fall by atomizing with a pure suspension of *Bacillus amylovorus* in sterile distilled water

In other experiments relating to the possibility of infection of the host tissues by *Bacillus amylovorus* without the intervention of wounds, the stamens and styles of open apple flowers, grown in the greenhouse under conditions designed to prevent injury by insects or other agencies, were removed with a pair of scissors and the receptacle cups filled with cocoa butter in such manner as to cover the nectaries and the wounds made incident to this excision. In all, 73 flowers were treated in this manner during the course of the experiment. After the cocoa butter had hardened, pure suspensions of *B. amylovorus* in sterile distilled water were sprayed upon the sepals and outer sides of the receptacle cups. The plants were then placed in the inoculation chamber for 24 hours, after which, in most instances, they were transferred to a chamber which was maintained at 28° C. and 70 per cent relative humidity. There they were left during the remainder of the experiment. In a number of instances where flowers and young fruits were inoculated in this manner, brownish, slightly depressed lesions (fig. 11) developed on the sides of the fruits after a period ranging from 5 to 10 days following inoculation. *B. amylovorus* was success-

fully recovered from the invaded areas in all of the reisolutions attempted from these lesions. The margins of the diseased areas, in many instances, were extended until the entire fruit was invaded. The results of these studies are given in Table 8.

TABLE 8.—Results of inoculation experiments with *Bacillus amylovorus* on apple flowers from which the stamens and styles were removed and the receptacle cups filled with cocoa butter before inoculation, Madison, Wis., 1928

Experiment No.	Period flowers were in moist chamber		Total flowers inoculated	Results of inoculation	
	Before inoculation	After inoculation		Plants remaining healthy	Diseased plants
	Hours	Hours	Number	Number	Number
1.....		24	67	35	32
		23	2	0	2
2.....	15	48	2	1	1
	24	23	2	0	2

Field studies of the infection of unopened blossoms by *Bacillus amylovorus* were also made in 1927 and 1928. A number of blossom buds in the early and middle closed-cluster stages of development were sprayed with nicotine sulphate (1-800) to kill any aphids which might be present. Later in the day, and, in some instances, on the following day, these unopened blossoms were sprayed with a pure suspension of *B. amylovorus* in sterile distilled water. These inoculated unopened blossoms were then caged in celluloid chambers in such a manner as to prevent the parts from coming in contact with any surface which might injure them. The results of these experiments are given in Table 9.

TABLE 9.—Results of inoculations made by spraying *Bacillus amylovorus* ^a on unopened blossoms, ^b Gays Mills, Wis., 1927-28

Date of inoculation	Variety inoculated	Inoculum applied with—	Blossom buds ^b inoculated	Number blighted	Per cent blighted
1927			Number		
May 4.....	Fameuse.....	Camel's-hair brush.....	14	3	21
May 13.....	Transcendent.....	Atomizer.....	130	31	24
May 16.....	McIntosh.....	do.....	37	0	0
May 20.....	Wealthy.....	do.....	23	0	0
May 25.....	Fameuse.....	do.....	154	4	3
1928					
Apr. 4.....	Transcendent.....	do.....	295	102	34
Apr. 6.....	do.....	do.....	82	42	51
Apr. 11.....	McMahon.....	do.....	60	0	0
Apr. 15.....	McIntosh.....	do.....	60	0	0
Apr. 23.....	Wealthy.....	do.....	17	0	0
Apr. 24.....	McIntosh.....	do.....	42	0	0
Apr. 26.....	do.....	do.....	50	0	0
Apr. 28.....	do.....	do.....	46	1	2
May 1.....	Wealthy.....	do.....	17	11	64
May 3.....	McIntosh.....	do.....	20	4	20
May 4.....	Wealthy.....	do.....	13	0	0
Do.....	McIntosh.....	do.....	47	4	9
May 5.....	Wealthy.....	do.....	10	2	20
May 6.....	McIntosh.....	do.....	12	0	0

^a The inoculum consisted of pure suspensions of *Bacillus amylovorus* from broth cultures in sterile distilled water.

^b The blossom buds inoculated ranged from those in the green tip to those in the open-cluster stage of development.

In field experiments carried on in 1928 unopened blossoms and young shoots, after a preliminary spraying with Derrisol (1-800) to kill any aphids which might be present, were given premoist treatments in specially constructed metal chambers mounted on tripods and lined with moist absorbent cotton. (Fig. 12.) A pure suspension of *Bacillus amylovorus* in sterile distilled water was then sprayed upon the young shoots and unopened blossoms and the inoculated parts placed in moist chambers in such a manner as to prevent injuries to the tissues. The results of these studies are given in Table 10.

TABLE 10.—Results of spraying *Bacillus amylovorus* ^a on unopened blossom buds and young shoots ^b which were subjected to moist treatments before and after inoculations, Gays Mills, Wis., 1928

Parts inoculated and dates of inoculation	Variety inoculated	Period in moist chamber		Total inoculated	Number blighted	Per cent blighted
		Before inoculation	After inoculation			
		Hours	Hours	Number		
Blossom buds: ^c						
Apr. 23.....	Wealthy.....		48	6	0	0
Apr. 24.....	Dudley.....	18	24	20	0	0
Do.....	McIntosh.....	18	24	20	0	0
Apr. 26.....	do.....	24	24	15	0	0
Do.....	Dudley.....	24	24	12	0	0
Do.....	McMahon.....	24	24	13	0	0
Apr. 28.....	do.....	48	48	11	0	0
Do.....	McIntosh.....	24	24	36	0	0
May 1.....	do.....	46	24	13	4	30
Do.....	Wealthy.....	46	24	21	7	33
May 3.....	McIntosh.....	24	24	8	7	88
Do.....	do.....	48	48	17	2	12
Do.....	Wealthy.....	24	24	22	4	18
May 5.....	do.....	24	24	21	5	24
Do.....	McIntosh.....	24	24	8	7	88
May 6.....	do.....	24	24	10	1	10
May 7.....	do.....	24	24	4	2	50
Do.....	Wealthy.....	24	24	21	4	19
May 9.....	do.....	24	24	14	8	57
Twigs: ^d						
May 8.....	do.....		24	10	6	60
May 10.....	do.....	48	24	5	3	60
May 11.....	do.....		24	15	7	47
May 14.....	Wealthy.....		24	10	0	0
Do.....	Transcendent.....		24	9	0	0
May 16.....	do.....			37	1	3
Do.....	Wealthy.....		24	5	1	20
Do.....	do.....			11	3	27
May 17.....	do.....			24	4	17
Do.....	Transcendent.....			18	0	0
May 18.....	do.....		24	7	0	0
Do.....	do.....	24	24	20	1	5
Do.....	Wealthy.....	24	24	20	0	0
May 20.....	do.....	24	24	7	1	14
Do.....	McMahon.....		24	9	0	0
May 21.....	do.....	8	24	8	0	0
May 24.....	Wealthy.....	24	24	4	0	0

^a The inoculum consisted of pure suspensions of *B. amylovorus* from broth cultures in sterile distilled water.

^b The parts inoculated were grown under conditions designed to minimize the chance of injury by insects or other agencies as described above.

^c The blossom buds inoculated ranged from those in the green-tip to those in the open-cluster stage of development.

^d The young shoots inoculated were from 2 to 7 inches long.

As is shown in Tables 9 and 10, the percentage of positive results was much more variable in the field experiments than in the greenhouse tests where conditions could be controlled more satisfactorily. Positive results were sufficiently numerous, however, to give strong indica-

tion that, under field conditions, *Bacillus amylovorus* can gain entrance to the host tissues through natural avenues of entry other than those of the inner (ventral) surface of the blossoms.

In an effort to ascertain more definitely the mode of entry of *Bacillus amylovorus* under the conditions of these tests, a detailed histological study was made of the tissues of young leaves and unopened blossoms which had been inoculated in the greenhouse by spraying with a pure suspension of *B. amylovorus* in sterile distilled water. Cases of stomatal penetration were found in tissues taken from the dorsal side of inoculated young apple leaves (fig. 13, A, and fig. 14, B) and from the sepals of inoculated unopened apple flowers. (Fig. 13, D.) The characteristic marginal invasion of young leaves suggests that the bacteria may gain access to the host tissues also through water pores, although the writer has thus far failed to find the bacteria entering these natural openings. The data which have been reported justify the conclusions: (1) That *B. amylovorus* can, under suitable conditions, infect young shoots and unopened blossoms of apple without the intervention of wounds, and (2) that, under some conditions, *B. amylovorus* can cause infection following penetration through stomata.

INTO OPEN FLOWERS

Following the work of Waite (30, 31), it has been generally accepted that *Bacillus amylovorus* gains access to the tissues of the open blossoms through the nectaries in the receptacle cup. So far as the writer is aware, however, no histological study has ever demonstrated

their actual entry into the tissues through the nectaries. It seemed desirable, in view of the lack of definite histological evidence, to trace the avenue of entry into the tissues of open blossoms. In these studies pure broth cultures of *B. amylovorus* were sprayed into the receptacle cups of a number of open apple and pear flowers. Fixations were made in formal acetic alcohol at short intervals after inoculation. The flowers were sectioned, after embedding in paraffin, and stained with rose bengal and light green. In both apple and pear flowers, which had been inoculated 30 hours before fixations were made, the bacteria were found gaining access to the host tissues through openings in the receptacle cups which appear to be stomata. (Fig. 13, B and C; fig. 14, A.) In none of the flowers sectioned by the writer thus far have the bacteria been found gaining access to the tissues of open flowers except by entering stomata. The writer has been unable to find evidence in the literature (see Eames and McDaniels (9, p. 78)) or in his own preparations that the floral nectaries

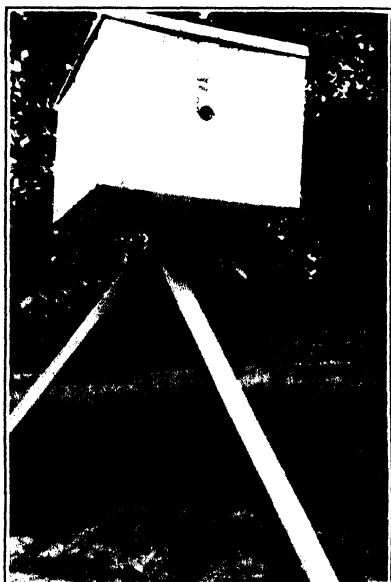


FIGURE 12.—Inoculation chamber used in the field experiments; the metal box was lined with moist absorbent cotton.

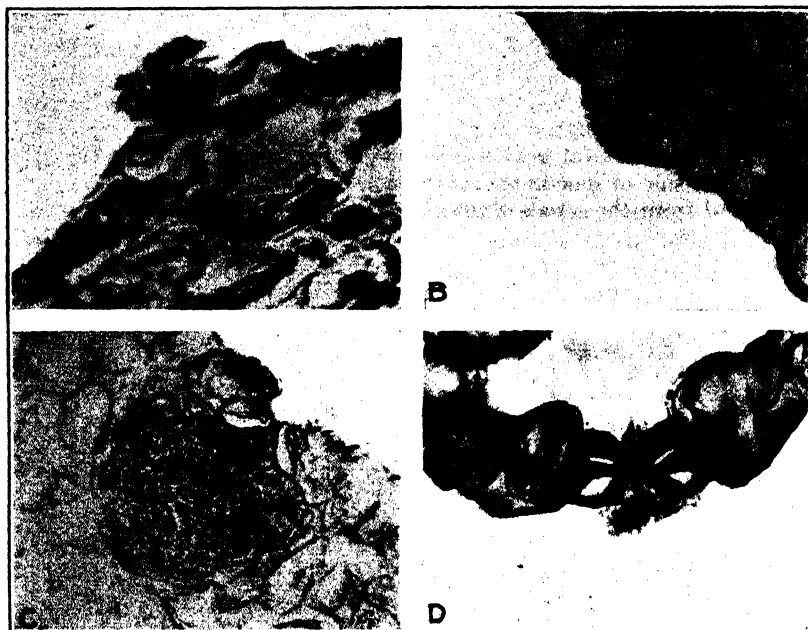


FIGURE 13.—Photomicrographs of host tissues invaded through stomata by *Bacillus amylovorus*. The inoculations were made by atomizing a pure suspension of the blight bacteria in sterile water. The bacteria are shown in the stomatal openings and in the substomatal chambers. A, Cross section of an apple leaf; B, longitudinal section of the receptacle cup of an apple flower; C, cross section of the receptacle cup of a pear flower; D, cross section of a sepal of an unopened apple flower

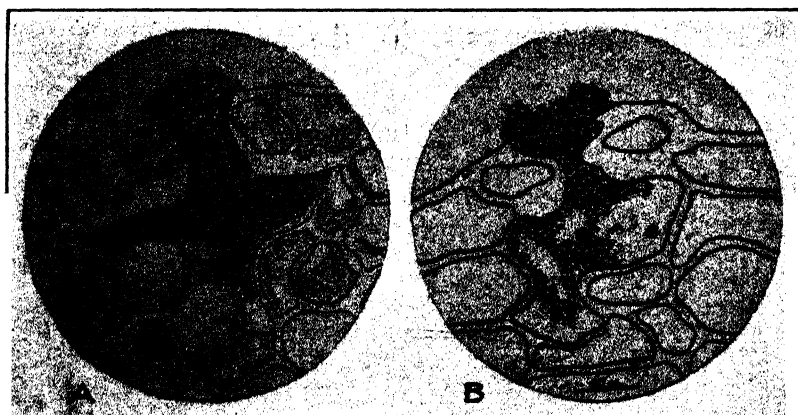


FIGURE 14.—Camera lucida drawings of host tissues invaded through stomata by *Bacillus amylovorus*. A, From the section shown in Figure 13, B; B, from the section shown in Figure 13, A

of apple or pear offer an open avenue for the entry of bacteria, as no intercellular spaces which pass from the surface through this tightly compacted layer have been demonstrated.

THE MIGRATION AND PARASITISM OF *BACILLUS AMYLOVORUS*

A number of valuable studies have been made of the relation of *Bacillus amylovorus* to its host. As most of these studies are readily accessible through the works of Stewart (28), Bachmann (4), Nixon (21), and Haber (11), an extended review of this literature appears to be unwarranted. However, notwithstanding the many excellent contributions which have been made to the knowledge of the intimate relationships existing between the causal organism and its hosts, many problems still remain unsolved. It is not at all clear just how the bacteria cause the death of the host cells. Do the bacteria secrete toxins or is the death of the cells due to the plasmolysis of the protoplast following the extraction of water from the cell? Still less is known as to how the bacteria gain access to the cells and how they migrate from one cell to another. The conditions surrounding the formation and extrusion of the well-known bacterial exudate have never been adequately described. Furthermore, considerable question still attaches to certain details of the mode of overwintering of the bacteria. While a comprehensive study of these problems could not be included in the present work, some attention was given them in the hope of contributing to their advancement, rather than their full solution.

STUDIES RELATING TO THE MANNER IN WHICH THE HOST CELLS ARE KILLED

A study of the literature reveals two views as to the manner in which the host cells are killed. Bachmann (4), Nixon (21), and others think that the cells are probably killed by the extraction of water from them and its passage into the intercellular spaces, with consequent plasmolysis. Stewart (28), however, is of the opinion that toxic products may be secreted by the pathogene which upon diffusion kill the cells.

The results of histological studies conducted by the writer accord with those of Bachmann (4) and Nixon (21) in showing that in early stages of migration the bacteria in the intercellular spaces were often found completely surrounding host cells without any noticeable ill effects other than a slight shrinking of the cell contents. Additional support to the view that plasmolysis following loss of water is an important factor in the death of the host cells was gained from experiments set up to determine whether the bacteria could thrive in solutions which had sufficient osmotic pressure to kill the host cells. A sucrose solution having an osmotic pressure of 20.73 atmospheres was made up and divided into two portions. Free-hand razor sections from a young apple leaf were placed in one of the portions and bacteria from a 3-day-old potato dextrose culture of *Bacillus amylovorus* in the other. At intervals microscopic examinations were made of the cells in the sugar solution. After remaining in the solution for one hour almost every cell in the section was plasmolyzed. The cells in control leaf sections in sterile distilled water, on the other hand, were turgid. Dilution plates were poured from the bacterial suspension in the sugar solution, and a large number of bacterial

colonies appeared on the agar plates after a suitable incubation period. There were live bacteria in the sugar solution even after a 24-hour exposure. This evidence indicates that the fire-blight bacteria can exist in the intercellular spaces in solutions having osmotic pressures sufficient to plasmolyze the living host cells.

In experiments conducted to determine whether toxins are produced by *Bacillus amylovorus*, Arthur (3) failed to detect the presence of toxic substances in filtered culture solutions in which the fire-blight bacteria had been growing. As further experimental evidence of the production or nonproduction of toxins by *Bacillus amylovorus* seemed desirable, the writer attempted to isolate toxic substances from (1) diseased host tissues and from (2) culture solutions in which the bacteria had been growing. Twigs affected with fire blight were ground up and the juice was extracted from the crushed tissue by squeezing through a cheesecloth. The juice was then filtered through a Berkefeld filter, and cross sections from a surface-sterilized apple leaf cut with a flamed razor were placed aseptically in (1) the filtrate and (2) sterile distilled water. At approximately 12-hour intervals, sections in the filtrate and in the controls were removed and examined under the microscope. No appreciable difference in the material from the filtrate and from sterile distilled water was noticed even after a 48-hour exposure. No evidence of toxic action was observed.

The possibilities of the production of exo-toxins which are precipitable by alcohol was next considered. In these studies 95 per cent alcohol was added to an 8-day-old nutrient broth culture and the resulting precipitate collected on filter paper, dried under aseptic conditions, and redissolved in sterile distilled water. This solution was then studied for toxic action as outlined above. No toxic action was detected. These experiments were repeated with the same results. This study suggests, therefore, that toxins of the sort which can be isolated by the methods used are not produced by *Bacillus amylovorus*.

STUDIES OF THE MIGRATION OF BACILLUS AMYLOVORUS IN THE HOST TISSUES

There has been some difference of opinion concerning the mode of migration of *Bacillus amylovorus* through the host tissues. Stewart (28), Bachmann (4), and certain other investigators seem to think that the bacteria migrate in the intercellular spaces in a free-swimming condition. Nixon (21) and Haber (11), however, take the view that they migrate through the tissues as zoogloea. Nixon (21) states that these zoogloea consist of jellylike matrices with blunt, more or less rounded ends, densely packed with bacteria. He further states that they migrate by a growth movement which parallels in many respects the movement of pseudoplasmodia of the Myxobacteriaceae.

In order to gain more evidence regarding the mode of migration of *Bacillus amylovorus* in the host tissues histological studies were made. A number of young shoots on potted Wealthy apple trees were inoculated in the greenhouse by puncturing near the tips with a fine needle dipped in a broth culture of the pathogene. The inoculated trees were placed in a moist chamber in the greenhouse at 25° C., and fixations were made in Flemming's strong fixing fluid at intervals of 1, 3, 5, 10, and 24 hours, respectively, after inoculation. Histological studies were then made of sections from this material, using Flemming's triple stain. In the twigs fixed 1 and 3 hours, respectively,

after inoculation the bacteria were found in the intercellular spaces, but no evidence was obtained to indicate that they were embedded in a jellylike substance. Their distribution and appearance suggested that they were in a free-swimming condition when the fixations were made. In the twigs fixed 10 and 24 hours, respectively, after inoculation, however, the bacteria were found in the intercellular spaces of the cortex embedded in a substance which stains differently than do the bacteria. Nixon (21) interprets these masses as zoogloea. Such interpretation would seem to depend on one's conception of zoogloea. This conception, as usually defined by bacteriologists, provides that the slimy matrix in which the bacteria are embedded is secreted by the bacterial cells. The evidence thus far adduced has not shown conclusively that, in the case of the fire-blight organisms in the host tissues, the matrix under discussion is of bacterial origin. Arthur (3), in his studies on the biology of pear blight, illustrates zoogloea formations. These so-called zoogloea were probably due to the presence of other organisms in his cultures, as Snyder (25), Stewart (28), and others have since failed to demonstrate the production of zoogloea by *Bacillus amylovorus* in pure culture. Until further evidence to the contrary is adduced, the possibility seems to remain that the fire-blight bacteria, upon being introduced through wounds into susceptible host tissues, first migrate in a free-swimming condition and later consolidate as they increase in numbers. Further study of the detailed mode of migration of the organisms and of the origin of the matrix in which they lie seems desirable.

Histological evidence suggests the production of enzymes and their activity in the development of the pathological condition. In stained sections of young apple shoots and flowers inoculated with *Bacillus amylovorus* the intercellular spaces invaded by the bacteria were, as a rule, larger than those in the corresponding normal tissues. In many instances bacteria were observed in invaded tissue completely surrounding the cells, which would seem to indicate that the substances which cement the cells together had been dissolved. Such evidence suggests that an enzyme of the nature of pectinase is produced either by *B. amylovorus* or by the host cells under the stimulus of bacterial invasion. However, the fact that no decided softening of the tissues ensues would suggest that any such enzymatic action occurs on a very small scale.

In histological studies of blight cankers the bacteria were found migrating from one cell to another through openings in the cell walls (fig. 15) which appear to have been formed by a dissolution of the wall substances. The portion of the wall which appears to



FIGURE 15.—Photomicrograph of a group of infected phloem cells in an active blight canker; the bacteria are shown within the cells and appear to have migrated from one cell to another through an aperture in the cell wall

have been dissolved is, in some instances, quite limited and localized. This fact suggests a mass action of the bacteria. In certain invaded areas, however, as in the phloem, comparatively large cavities were found in which the bacteria were present in great numbers. Histological evidence suggests that a solvent action on cell walls played a part in the formation of these cavities. It seems probable that either the parasite or the host under its stimulus secretes very small amounts of an enzyme of the nature of cellulase.

Pressures which may develop in the intercellular spaces incident to the growth of the bacteria and to the physiological processes of host and parasite may possibly play some part in the development of the tissue changes which have just been discussed. However, the sharp localization of many of the changes observed and certain details of the histological picture, as orifices in cell walls bounded by well-rounded edges (fig. 15) and a scarcity of evidence of the crushing of cells, make it difficult to explain the observed phenomena wholly on the basis of pressures developed outside of the host cells.

Experiments were made with pure cultures of *B. amylovorus* with the aim of isolating any exo-enzymes which are precipitable by alcohol. In these experiments 95 per cent alcohol was added to an 8-day-old nutrient broth culture of *B. amylovorus* and the resulting precipitate collected on filter papers, dried under aseptic conditions, and redissolved in sterile distilled water. Pieces of carrots cut aseptically from carrot roots were dropped into the redissolved precipitate and at the same time other pieces of carrots were placed in tubes of sterile distilled water as controls. At approximately 24-hour intervals, microscopic examinations were made of thin razor sections from the carrot tissues in the redissolved precipitate and from those in the controls. After 24 hours had elapsed no significant difference could be detected between the sections in the redissolved precipitate and the controls in sterile distilled water. Differences were discernible, however, after 72 hours of exposure. The cell walls of the carrot tissue exposed to the action of the redissolved precipitate were more swollen and the intercellular spaces appeared to be larger than those in the carrots immersed in sterile water. This would seem to indicate that the pectic layers were being slowly dissolved by enzymes which had been secreted by the bacteria. The fact that this action is so slow, however, suggests that only small amounts of exo-enzymes precipitable by alcohol are secreted by *B. amylovorus*. There is no evidence of a rapid dissolving action as is the case with the soft-rot organism, *B. carotovorus*.

An attempt was also made to ascertain whether cellulase is produced by pure cultures of *B. amylovorus*. In these studies pure cultures of the pathogene were grown on cellulose agar for a period of 10 days. There was no indication that the cellulose was being fermented by the bacteria. Furthermore, no action was noticeable on pieces of filter paper even after a 30-day exposure to the action of a nutrient broth culture of *B. amylovorus*. The results of these studies suggest that cellulase is not produced by *B. amylovorus* in significant amounts in cultures of the kind used in these studies. However, there still remains the possibility that the pathogene produces an enzyme of the nature of cellulase in association with the host. It may also be that cellulase is not secreted by *B. amylovorus*, but is produced by the host cells under the stimulus

of bacterial invasion. Unless the production of small amounts of some cell-wall dissolving substance in the invaded tissues either by the host or the parasite is postulated, the evidence presented by histological studies of the diseased tissues is without adequate interpretation.

VARIETAL RESISTANCE OF APPLES TO FIRE BLIGHT

No variety of apple grown in Wisconsin has been found to be totally immune to fire blight. However, it has long been observed that some varieties suffer much less than others from this disease. This was shown rather strikingly in studies of the relative abundance of blossom infections in a number of different apple varieties growing in the orchards at Gays Mills. Based on counts made in 1926, 1927, and 1928, Transcendent, McMahon, Fameuse, and Wealthy are more subject to blossom-blight attacks, under Wisconsin conditions, than McIntosh, Northwestern Greening, or Dudley. (Table 11.) It is a rather significant fact that those varieties which are most subject to blossom infection have, as a rule, the most overwintering sources of primary inoculum. (See Table 1, p. 584.)

TABLE 11.—*The per cent of blossom blight on some apple varieties, Gays Mills, Wis., 1926-1928*

Year and variety	Blossom clusters a—		
	Exam- ined (number)	Blighted	
		Number	Per cent
1926			
Transcendent.....	925	228	24
Fameuse.....	24,805	5,055	21
Wealthy.....	46,182	3,225	7
Northwestern Greening.....	2,112	64	3
McIntosh.....	1,208	28	2
1927			
McMahon.....	19,696	3,949	20
Fameuse.....	19,205	1,816	9
Wealthy.....	19,416	1,364	7
Dudley.....	12,107	363	3
McIntosh.....	19,620	561	3
Northwestern Greening.....	13,013	333	3
1928			
Fameuse.....	16,000	420	3
Wealthy.....	16,000	381	2
McIntosh.....	16,000	382	2
Dudley.....	16,000	204	1
Northwestern Greening.....	16,000	94	1

* The blossom clusters were examined the third and fourth weeks after petal fall. The method used for determining the percentage of blossom blight consisted in examining from 50 to 100 blossom clusters representatively distributed in all parts of the tree in each of approximately 100 representative trees of each variety.

INFLUENCE OF POLLINATION ON THE SUSCEPTIBILITY OF FLOWERS

Gossard and Walton (10) state that fertilized blossoms become resistant to infection by *Bacillus amylovorus* sooner than do unfertilized ones. Their experiments led them to conclude that blossoms which have been pollinated for 72 hours are not likely to be infected by the fire-blight organism and that susceptibility to infection does not

exist in blossoms that have been pollinated for 144 hours. They took the view that a host of bees by virtue of their pollinating activity would in reality be "friendly little sprites," transforming susceptible flowers into resistant ones by early pollination instead of being dreaded disseminators of blight as they are commonly supposed to be.

To gain further evidence of the possible influence of pollination on the susceptibility of flowers to infection by *Bacillus amylovorus*, studies were carried on in the field during the blooming seasons of 1926 and 1927. Inasmuch as practically all varieties of apples are self-sterile, it was not deemed necessary to emasculate the flowers, which was the procedure followed in Gossard and Walton's experiments. In the writer's experiments a number of flower clusters, both terminal and lateral, on several varieties of apples were inclosed in parchment paper bags before any of the blossoms had opened, so as to prevent natural pollination. When the blossom had opened, freshly collected pollen from other varieties was applied to the stigma of each flower by means of a camel's-hair brush. Other flowers were left unpollinated. At stated intervals after pollination inoculations were made by spraying pure broth cultures of *Bacillus amylovorus* into the receptacle cups by means of an atomizer. Unpollinated flowers were inoculated at the same time in like manner. The results of these studies are given in Table 12. While these data are not inclusive they suggest that the flowers remain susceptible to infection much longer after pollination than appeared from the experiments of Gossard and Walton.

TABLE 12.—The influence of pollination on the susceptibility of flowers of some varieties of apples to infection by *Bacillus amylovorus*, Gays Mills, Wis., 1926

Year and variety	Interval between pollination and inoculation	Pollinated clusters—		Unpollinated clusters—	
		Inoculated	Blighted	Inoculated	Blighted
	Hours	Number	Per cent	Number	Per cent
1926					
Dudley.....	69	6	100		
Do.....	114	6	100	10	100
Do.....	144	5	40	5	60
McMahon.....	70	4	100		
Do.....	122	2	100	4	100
Do.....	188	3	0		
Fameuse.....	50	8	100	5	100
Do.....	120	2	100	15	46
Do.....	122	7	71		
Do.....	168	5	0		
1927					
Northwestern Greening.....	191	16	62	9	88
Do.....	209	11	18	13	0
Do.....	216	11	0	11	0
McIntosh.....	118	4	100	4	0
Do.....	161	10	90	6	0
Do.....	213	10	0	12	0
Do.....	216	9	0	11	0
Fameuse.....	166	4	100		
Do.....	189	8	25	10	20
Do.....	211	3	0	9	0
Wealthy.....	187	5	100	4	0
Do.....	258	6	50		
Do.....	265	5	0		

DURATION OF SUSCEPTIBILITY OF YOUNG APPLE FRUITS

During the course of studies of the epidemiology of fire blight in the field in 1927 results were obtained which seemed to suggest that young apple fruits were susceptible to infection for some time after petal fall. (Fig. 2.) As experimental evidence seemed desirable, studies were conducted in the greenhouse in the spring of 1928, using potted dwarf Yellow Transparent apple trees. To prevent injury by aphids these trees had been sprayed with Derrisol (1-800) at intervals from the time the buds began to open until the blossoms opened. Pure suspensions of *Bacillus amylovorus* in sterile distilled water were sprayed at approximately daily intervals into the receptacle cups and upon the outer parts of successive series of young fruits which had previously been pollinated. The trees bearing the inoculated fruits were placed in the moist chamber for 24 hours after inoculation. They were incubated at 28° C. and 70 per cent humidity. The results of this study appear in Table 13. Field studies of the duration of susceptibility of young apple fruits to infection were also carried on in 1928. The young apples were sprayed with Derrisol (1-800) to kill any aphids which might be present, and at varying intervals after petal fall a pure suspension of *B. amylovorus* in sterile distilled water was sprayed into the receptacle cups and upon the outer parts of the young apples. However, as it was impossible to control the temperature and humidity following inoculation in the field, the results of these trials are not as striking as those obtained in the greenhouse. (Table 13.) While these studies need to be extended, the results indicate that, under favorable conditions, young apple fruits may remain susceptible to infection without the intervention of wounds for at least a week after petal fall.

TABLE 13.—Results of inoculating young apple fruits, grown under conditions designed to prevent injury by insects or other agencies, with *Bacillus amylovorus*^a at varying intervals after petal fall, 1928

Location and variety inoculated	Number of days after petal fall on which inoculation was made	Fruits inoculated (number)	Fruits blighted	
			Number	Per cent
Greenhouse:				
Yellow Transparent.....	0	10	10	100
Do.....	2	30	30	100
Do.....	3	20	20	100
Do.....	4	9	8	89
Do.....	5	17	15	88
Do.....	6	20	15	75
Do.....	7	20	8	48
Do.....	10	11	10	90
Orchard:				
Fameuse.....	1	30	30	100
Do.....	2	21	21	100
Do.....	3	27	0	0
Do.....	4	29	0	0
Do.....	5	40	0	0
Do.....	6	30	0	0
Do.....	7	25	0	0
Do.....	10	36	0	0
Do.....	15	45	0	0

^a The inoculum consisted of a pure suspension of *B. amylovorus* in sterile distilled water.

PRELIMINARY STUDIES ON THE NATURE OF RESISTANCE TO FIRE BLIGHT

Although the resistance of certain varieties of apples and pears to infection by *Bacillus amylovorus* has been noted by many investigators (Waite (31), Crandall (8), Jones (17), Whetzel and Stewart (35), Stewart (28), Hansen (12), Reimer (22, 23), and others), comparatively little attention has been given to a consideration of the nature of this resistance. Hewitt (15), after a series of chemical analyses, concluded that an index to resistance and susceptibility is to be had in the ordinary analysis for starch. He states that those varieties which gave the higher percentages of starch upon analysis are more susceptible to injury by the blight organism. Stewart (28) attributes differences in varietal resistance to character of growth rather than to the presence of any inherent qualities which tend to make the trees immune. He says:

The young tender tissue of the Douglass variety (a variety of pear which is presumably quite resistant to fire blight) is apparently as susceptible as that of any other pear but the tendency for the tissue to rapidly harden, soon after it is formed, impedes the activities of the blight bacteria and the injury is less severe.

Higgins (16) is of the opinion that the formation of gummy substances in the host tissues in response to bacterial invasion is responsible for resistance, as masses of gum were found in the border region between the living and dead tissues of a supposedly resistant variety of pear.

No variety of apple grown in Wisconsin has been found to be totally immune to fire-blight attacks. Even in varieties such as the Northwestern Greening and McIntosh, which commonly seem to be quite resistant, one occasionally finds a tree which is heavily infected. In 1927 several McIntosh trees on which more than 50 per cent of the flowers were diseased were found in the orchards at Gays Mills, although on the majority of the trees of this variety less than 3 per cent of the blossoms were blighted. (Table 11.) However, in those instances where blossom and twig blight were severe in trees of a resistant variety, it is a significant fact that in fully 95 per cent of the blighted twigs and diseased clusters examined only the current year's growth seemed to be invaded. Higgins (16) noticed a similar condition in the pear, for he reports that flower infection seems to occur as readily in resistant varieties as in the less resistant ones, but only a little damage is produced by each infection in the former. He states, furthermore, that in resistant varieties young twigs and flowers infected with fire blight are killed but " * * * infection in no case passed back into the mature wood of the previous season's growth."

In those cases where the pathogene had invaded the preceding year's growth the cankers formed on resistant varieties were quite superficial, the discoloration being limited in most instances to the cortex and the outermost part of the phloem. These cankers commonly cease enlarging soon after their formation and become delimited from the surrounding tissues by a peripheral cork barrier which gives a marked reaction with Sudan III. As the diseased tissue dries and shrinks a fissure usually develops along the boundary. In the susceptible varieties of apples, as the Fameuse and Transcendent, the organism does not commonly stop after reaching the older growth, but in most instances forms cankers or continues to invade the

branches, so that large limbs are often infected. In the cankers formed the discolored tissue, in a large number of instances, extends down to the cambial region. These cankers remain active for some time after their formation. It is significant that a much larger percentage of the cankers formed in the more susceptible varieties become holdovers than is the case in those formed in resistant varieties. (Table 1.) This difference in the degree to which invasion and subsequent inactivation take place in the resistant and the susceptible varieties seems to be correlated with the rapidity with which the host reacts to the invasion of the pathogene following infection. It would seem from these observations that the host offers specific resistance to the invasion of the pathogene in some instances and little or none in others.

STUDIES OF VARIABILITY IN RESISTANCE AND SUSCEPTIBILITY TO FIRE BLIGHT

In order to obtain more evidence relating to the nature of resistance to fire blight, a study of variability in varietal resistance or susceptibility to the disease was begun.

RELATION OF TEMPERATURE

Young shoots approximately 5 inches long grown on potted Wealthy (susceptible) and Northwestern Greening (fairly resistant) apple trees in the greenhouse were inoculated by inserting near the tip of the stem a needle which had been dipped in a pure broth culture of *Bacillus amylovorus*. Inoculated plants were then incubated in the chambers previously described (p. 596) at each of the following temperatures, 16°, 24°, and 28° C. The relative humidity at each of these temperatures was approximately 50 per cent. In the inoculated shoots which were incubated at 16° disease development was so slow in both varieties that manifestation of resistance or susceptibility was largely precluded. In each case only the apical portions of the shoots turned brown, there being no further extension of the disease down the stem. At 24° and 28°, however, the inoculated Wealthy shoots were completely blighted at the end of 10 days, whereas in the inoculated Northwestern Greening shoots the disease was limited to the apical parts of the twigs. In the inoculated Northwestern Greening shoots it was further observed that a stimulation of the buds in the axils of the remaining healthy leaves often took place and secondary shoots were produced. These observations would seem to indicate that within the range of conditions of this limited experimentation the resistance of the Northwestern Greening is not broken down or altered by variations in temperature.

RELATIONS OF MOISTURE

Young shoots grown on potted Wealthy and Northwestern Greening trees were inoculated by inserting near the tip of the stem a needle dipped in a pure broth culture of *Bacillus amylovorus*. They were then placed in a saturated atmosphere in an infection chamber.⁷ The temperature in this chamber was maintained at 26° C. Under these conditions the inoculated shoots of the Northwestern Greening were just as severely attacked as the Wealthy. At the end of five

⁷ This apparatus is described by Keitt and Jones (18).

days the inoculated Northwestern Greening twigs were completely blighted. Though it has not yet been feasible to conduct experiments concerning the possible influence of other factors, such as light and the type of vegetative growth of the experimental plants, it would appear probable from these results that resistance can be altered by subjecting so-called resistant varieties to the influence of humidity under suitable conditions of temperature and light. The reasons for this change in resistance to fire blight have not been fully analyzed as yet. However, a constantly maintained excess of water in the tissues would be expected to hinder the accumulation, oxidation, and condensation processes which are involved in the formation of the corky barrier which appears to limit the development of blight lesions under field conditions. Furthermore, it seems probable that an increase in the amount of moisture in the intercellular spaces would favor the growth and migration of the blight bacteria.

STUDIES OF METHODS OF CONTROL

Fire blight is one of the most difficult to combat of all of the diseases of pomaceous fruits. Arthur (3) suggested the cutting out of blighted parts as a control measure. Waite (33) and Smith (24) demonstrated that the excision method could be successfully employed if a sufficiently large area of the infested territory is adequately policed and the affected parts of the trees are promptly removed. Other methods of control have since been advocated, so that at present the control measures which are in vogue fall chiefly into five categories, as follows: (1) Ridding the trees of the sources of inoculum by excision methods, (2) control or elimination of insect carriers, (3) modification of the susceptibility of the host by appropriate cultural conditions, (4) spraying with Bordeaux mixture to reduce the number of blossom infections, and (5) the use of resistant varieties. In Wisconsin numerous attempts to control fire blight by the use of the commonly recommended methods have met with but indifferent success. The ineffectiveness of these measures in controlling the disease is particularly noticeable in years when epidemic outbreaks of fire blight occur. In view of the difficulties which have been encountered by the apple growers of this State in controlling fire blight it was deemed advisable to test experimentally the control measures now advocated and to seek to develop improved methods. Accordingly, several series of plots were marked out in the fall of 1925 at Gays Mills, Wis. and each plot was subjected to a special treatment. One plot was left untreated as a control. Counts were made after the blooming periods to determine the percentage of blossom blight developing in the different plots. Similar experiments were conducted in 1927 and 1928. The treatments and the results are shown in Table 14.

It is recognized that these experiments are not extensive enough to warrant definite conclusions, particularly in view of the small amount of disease which developed in most of the control plots. However, from these limited data the following conclusions seem to be warranted: (1) Excision seems to have led to some reduction in the amount of blight in the following season, but (2) spraying for the control of aphids did not result in any material reduction in the amount of blight. However, while excision reduced the number of infections in most of these plots, it has not fully controlled the disease. The infections in

the plots in which excision was practiced could be traced back, in most cases, to overwintering cankers and blighted twigs overlooked in the excision process. However, the writer exercised unusual care in the effort to accomplish thorough excision. It is scarcely to be expected that better results would be obtained in commercial orchard operations.

TABLE 14.—*Studies of the comparative value of various measures for the control of apple fire blight, Gays Mills, Wis., 1926-1928*

Year and series	Plot number	Variety	Treatment	Blossom clusters			
				Examined	Blighted		
				Number	Per cent		
1926							
Series I	1	Fameuse	Excision, September, 1925, and April, 1926	3,394	14		
			Scalecide (1-15), Apr. 30, 1926				
			Nicotine dust, May 3, 1926				
			Bordeaux, 2-4-50, June 23, 1926				
	2	do	Excision, September, 1925, and April, 1926	3,110	6		
	3	do	Scalecide (1-15), Apr. 30, 1926	2,755	16		
			Nicotine dust, May 3, 1926				
			Bordeaux, 2-4-50, June 23, 1926	5,551	33		
	4	do	Control				
Series II	1	Wealthy	Excision, September, 1925	8,044	8		
			Scalecide (1-100), May 5, 1926				
	2	do	Excision, September, 1925	8,672	5		
	3	do	Scalecide (1-100), May 5, 1926	9,762	7		
	4	do	Control	6,944	6		
1927							
Series I	1	Fameuse	Excision, August and September, 1926	4,990	3		
			Nicotine dust, May 1, 1927				
	2	do	Excision, August and September, 1926			4,022	4
	3	do	Nicotine dust, May 1, 1927			3,684	6
	4	do	Control	4,678	5		
Series II	1	do	Excision, August, 1926	4,682	2		
	2	do	Control	4,287	7		
Series III	1	do	Excision, August, 1926	3,127	1		
	2	do	Nicotine dust, May 1, 1927	1,730	0		
	3	do	Control	3,155	3		
	4	do	Nicotine sulphate (1-800), April, 1927	4,037	8		
Series IV	1	Wealthy	Excision, August, 1926	4,523	12		
	2	do	Nicotine sulphate (1-800), April, 1927				
	3	do	Control			4,403	14
1928							
Series I	1	Fameuse	Excision, August, 1927	2,045	1		
			Derrisol (1-800), Apr. 27, 1928				
	2	do	Excision, August, 1927			2,160	2
	3	do	Derrisol (1-800), Apr. 27, 1928			2,974	5
	4	do	Control	1,000	2		
Series II	1	do	Excision, August, 1927	2,475	0		
			Derrisol (1-800), Apr. 27, 1927				
	2	do	Excision, August, 1927			2,530	0
	3	do	Derrisol (1-800), Apr. 27, 1928			2,280	2
	4	do	Control	3,300	2		
Series III	1	do	Excision, August, 1927	1,915	1		
	2	do	Control	1,925	1		

* The unusually high blossom-blight count in this plot was due to a number of overwintering cankers which were overlooked in certain of the trees during excision.

BORDEAUX MIXTURE

Stevens, Ruth, and Peltier (26) and McCue (20) report that Bordeaux mixture applied during the blossoming period of pears seems to offer some protection against blossom blight. Reimer (22, 23) concluded after a series of experiments that Bordeaux mixture applied to pear trees during the open-cluster stage of blossom-bud development will materially reduce the number of blight infections but will not prevent all infections. Recently McCown (19) reports having re-

duced the number of blossom infections in apple by an application of Bordeaux mixture when the flowers were just coming into full bloom. In view of the fact that the inoculum for fire blight is largely rain-borne under Wisconsin conditions and that the critical period for infection includes the blossoming time, it was deemed advisable to determine whether an application of Bordeaux mixture at blossoming time would reduce the amount of infection in apples under local conditions. In 1926 Bordeaux mixture, 2-4-50, was applied to 40 Wealthy apple trees and 100 McMahons at full bloom, care being taken, in so far as it was possible, to cover the trees well. A month later blossom-blight counts were made in the sprayed and unsprayed plots. (Table 15.) In 1927 tests were again made, using a stronger solution of Bordeaux mixture in accordance with Reimer's (22, 23) recommendations. In this experiment Bordeaux mixture, 3-6-50, was applied to 80 Fameuse trees when most of the blossoms were just beginning to open. The results, which appear in Table 15, show that the amount of blossom blight was not materially reduced by these Bordeaux-mixture applications. However, more data are needed before any valid conclusions can be drawn as to the value of Bordeaux-mixture applications in the control of blossom blight under Wisconsin conditions.

TABLE 15.—*The effect of Bordeaux-mixture applications on subsequent blossom-blight development, Gays Mills, Wis., 1926-27*

Year and variety	Strength of Bordeaux mixture used	Sprayed blossom clusters		Unsprayed blossom clusters	
		Ex- amined	Blighted	Ex- amined	Blighted
1926 Wealthy..... McMahon.....	2-4-50	<i>Number</i> 7, 170	<i>Per cent</i> 2	<i>Number</i> 7, 045	<i>Per cent</i> 5
	2-4-50	7, 261	19	7, 352	17
1927 Fameuse.....	3-6-50	5, 887	14	6, 180	19

BACTERICIDES

In an attempt to find a suitable chemical treatment for the control of fireblight, laboratory and greenhouse tests were made. In the laboratory a number of different chemicals which were thought to possess bactericidal properties were tested. Among the substances tried were sodium silicofluoride, calcium silicofluoride, Bordeaux mixture, 4-4-50, Bordeaux mixture, 4-8-50, Uspulun,⁸ Semesan Bel,⁹ and Improved Semesan Bel.¹⁰ In testing the bactericidal action of these substances the method outlined by Anderson and McClintic (1), with some modifications, was followed. Very briefly, the procedure used was as follows: One-tenth of a cubic centimeter of a 24-hour-old nutrient broth culture of *Bacillus amylovorus* was transferred by means of a sterile pipette to a test tube containing 5 c. c. of a solution of suspension of the chemical to be tested. This tube was

⁸ A proprietary compound containing chlorophenol mercury.

⁹ A proprietary compound containing hydroxymercurichlorophenol.

¹⁰ An organic mercury compound.

shaken thoroughly and at the end of 2½, 5, and 10 minutes, respectively, a loopful (4 mm. in diameter) of the bacterial suspension in the solution being tested was transferred to a test tube containing 10 c. c. of sterile nutrient broth. The tubes were then incubated at 24° C. for 48 hours. Growth in the tubes indicated that the substances at the particular concentration used were ineffective in killing the bacteria. A duplicate set of controls was run for each chemical tested. As controls, one-tenth of a cubic centimeter of a 24-hour-old nutrient broth culture of *B. amylovorus* was added to 5 c. c. of sterile nutrient broth. A loopful of the resulting bacterial suspension was transferred to a sterile tube of nutrient broth at 2½, 5, and 10 minute intervals, respectively. Of the substances tested, sodium silicofluoride and calcium silicofluoride failed to show significant bactericidal action against *B. amylovorus*, as they were ineffective at all dilutions tried. (Table 16.) Bordeaux mixture, 4-4-50 and 4-6-50, were also ineffective. A 1 per cent solution each of Uspulun and Semesan Bel, a one-sixteenth per cent solution of Improved Semesan Bel, and Bordeaux mixture, 4-8-50, were all effective in inactivating the bacteria, as was indicated by the lack of growth in the tubes of broth. The control tubes on the other hand, were heavily clouded, indicating that in these tubes growth had taken place. The most promising of these chemicals were subsequently subjected to further tests. In these tests approximately one-tenth cubic centimeter of each of a one-half per cent solution of Improved Semesan Bel and Bordeaux mixture 4-8-50 was placed on a sterile glass slide and allowed to air dry under aseptic conditions.

TABLE 16.—The bactericidal action of certain chemicals on *Bacillus amylovorus* Madison, Wis., 1928

Chemical and concentration ^a	Results ^b after exposure for—			Chemical and concentration ^a	Results ^b after exposure for—		
	2½ min-utes	5 min-utes	10 min-utes		2½ min-utes	5 min-utes	10 min-utes
Sodium silicofluoride:				Calcium carbonate:			
1:20,000.....	+	+	+	1:10.....	+	+	+
1:1,000.....	+	+	+	Uspulun:			
1:100.....	+	+	+	1:200.....	+	+	—
Calcium silicofluoride:				1:100.....	—	—	—
1:1,000.....	+	+	+	Semesan Bel:			
1:200.....	+	+	+	1:500.....	+	+	+
1:100.....	+	+	+	1:200.....	+	+	+
Bordeaux mixture:				1:100.....	—	—	—
4-6-50.....	+	+	—	Improved Semesan Bel:			
4-8-50.....	—	—	—	1:800.....	—	—	—
Calcium hydroxide:				1:1,600.....	—	—	—
1:10.....	+	—	—				

^a The chemicals used, except proprietary products, were "chemically pure"; dilutions were made with sterile distilled water on the basis of weight.

^b + = growth in the tubes of broth; — = no growth in the broth

Approximately five-hundredths of a cubic centimeter of a pure suspension of *B. amylovorus* in sterile distilled water was then added to the surface of the dried film on the slide. At the end of 10 minutes' exposure a loopful of the bacterial suspension was transferred to several drops of sterile water in a sterile Petri dish to which corn-meal agar was added and mixed with the bacterial suspension by the

usual poured-plate technic. At the same time a loopful of the bacterial suspension from a control was transferred to (1) a Petri dish containing sterile water and to (2) a Petri dish containing a loopful of the sterile bactericide. After a suitable incubation period the number of colonies in each plate was determined. This experiment was repeated several times with consistent results, a typical series of which appears in Table 17. These data show that the highly alkaline Bordeaux mixture exercised a strongly toxic effect (fig. 16, B) on the blight bacteria, while Improved Semesan Bel, 1-200, (fig. 16, A) completely inactivated them under the conditions of these experiments.

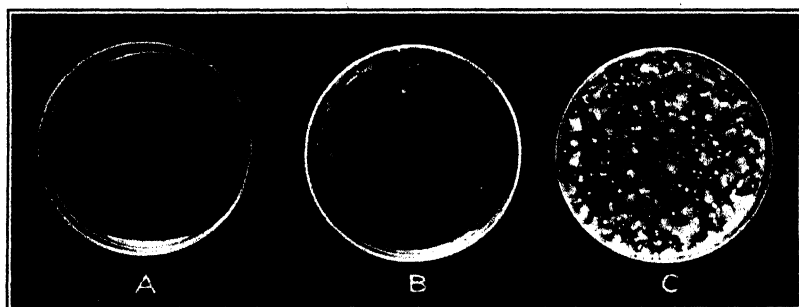


FIGURE 16.—Cultures showing the action of certain chemicals on *Bacillus amylovorus*: A, Bacteria exposed to the action of Improved Semesan Bel, one-half per cent, before plating; B, bacteria exposed to action of Bordeaux mixture, 4-8-50, before plating; C, control

TABLE 17.—The toxic effect of certain bactericides ^a on *Bacillus amylovorus*

Chemicals and concentration ^b	Average number of colonies per plate after 10 minutes exposure
Bordeaux mixture, 4-8-50.....	c 45
Improved Semesan Bel, 1:200.....	0
Untreated control.....	d +

^a These bactericides were placed on sterile glass slides and allowed to become air dry under aseptic conditions; their action upon *B. amylovorus* was then tested.

^b Dilutions were made with sterile distilled water on the basis of weight.

^c The colonies which developed were mostly small, submerged, and somewhat abnormal in appearance.

^d Colonies too numerous to count.

Tests of the effectiveness of Semesan Bel in reducing the amount of fire blight under field conditions were conducted in the spring of 1927. The limbs and trunks of 16 Fameuse apple trees which contained numerous hold-over cankers and twigs were thoroughly sprayed with a 2 per cent solution of Semesan Bel on April 20, when the blossom buds were in the early closed-cluster stage of development, and again five days later. No further applications were made, due to the limited amounts of the spray material on hand. Blossom-blight counts made later in the season failed to show any significant difference in the development of the disease on the sprayed and the unsprayed trees. (Table 18.) In 1928 further tests were made, using Improved Semesan Bel and several other organic mercury

compounds. In some instances as many as five applications were made, starting when the buds were in the early closed-cluster stage of development and continuing at intervals until petal fall. In some cases (Table 18) it would seem that the number of blight infections was reduced by certain of these chemicals. However, in interpreting these data it should be remembered that the number of overwintering sources of primary inoculum varied greatly in different trees. There seems to be considerable promise, nevertheless, in some of the chemicals tried. More data are necessary before conclusions can be drawn.

TABLE 18.—*Effect of certain chemical treatments on subsequent blight development on Fameuse apple, Gays Mills, Wis., 1927 and 1928*

Year and number of trees in plot	Treatment used	Dates of application	Stage of bud development ^a	Sprayed blossom clusters		Unsprayed blossom clusters	
				Total	Blighted	Total	Blighted
1927				Number	Per cent	Number	Per cent
16.....	Semesan Bel, 2 per cent.....	Apr. 20 Apr. 25	GT ECC	862	20	1,053	15
1928							
10.....	Semesan Bel, $\frac{1}{8}$ per cent.....	Apr. 21 do.....	GT GT	1,010	2	1,000	8
20.....	do.....	Apr. 26 May 6 May 11 May 19	ECC MCC OC PF	1,010	8	1,000	7
12.....	Semesan Bel, 1 per cent.....	May 6 May 11 May 19	MCC OC PF	1,020	4	1,000	8
1.....	K-1-GB, ^b 1 per cent.....	May 1 May 6 May 11 May 19	ECC MCC OC PF	385	6	618	3
8.....	K-1-CB, 1 per cent.....	May 3 May 6 May 19	ECC MCC PF	525	3	1,578	9
8.....	K-1-FB, 1 per cent.....	May 3 May 6 May 19	ECC MCC MCC	745	1	1,578	9

^a GT=Green tip; ECC=early closed cluster; MCC=middle closed cluster; OC=open cluster; PF=petal fall.

^b K-1-GB, K-1-CB, and K-1-FB are organic mercury compounds.

SUMMARY

In Wisconsin, epidemic outbreaks of fire blight of apple are frequent and very destructive. Numerous attempts of orchardists to control the disease by the commonly recommended methods have been attended with but indifferent success. The variability in the severity of occurrence of fire blight and the difficulty of its control have led to further studies of the disease under local conditions. Two correlated lines of work have been undertaken: (1) Studies of the development and control of the disease in relation to the natural environment and (2) detailed studies, under partly controlled conditions in the laboratory and greenhouse, of certain phases of the life history of the casual organism in relation to pathogenesis.

Records of the seasonal development of the host, the pathogene, and the disease at Gays Mills, Wis., are presented for the years 1926, 1927, and 1928 in correlation with certain meteorological data. Although no general epidemic outbreaks of fire blight occurred in these years, local outbreaks were prevalent. These outbreaks were traceable to local sources of primary inoculum and appeared to be corre-

lated more with the amount and distribution of rainfall and suitable temperatures for infection than with insect activity.

Bacillus amylovorus was found to overwinter in the apple in Wisconsin in association with cankers and blighted twigs. While only a small percentage of the cankers or blighted twigs formed in any one year carried the blight organisms over the winter, it was shown that the bacteria live over winter under Wisconsin conditions in sufficient quantity to furnish a plentiful source of inoculum for primary infection. Varieties were found to differ greatly in the percentage of blighted twigs and of cankers which carry the blight bacteria through the winter. Of the varieties studied, the percentage of cankers in which the organism passed the winter was greatest in Transcendent, Yellow Transparent, Fameuse, and Wealthy. On the Dudley, McIntosh, and Northwestern Greening, on the other hand, comparatively few hold-over lesions were found. The smooth-margined type of lesion was largely responsible for carrying the disease over winter. The delimited lesion was commonly found to be inactive.

Isolation experiments furnished further evidence that the blight organisms overwinter largely in the apparently healthy tissues immediately adjacent to the discolored margin of the canker. As 33 attempts made to isolate the bacteria from normal tissues 2 inches from the macroscopic margins of cankers were all negative, it appears unlikely that the blight bacteria often overwinter more than 1 inch from the visible margin of the canker.

Meteoric water was found to be the most important agent concerned in Wisconsin in the spread of the primary inoculum from hold-over cankers and twigs where the pathogene overwinters to the current year's growth.¹¹ Observations have failed to indicate that pollinating or other insects are concerned to any significant extent in the spread of the bacteria from overwintering sources to the susceptible parts.

Meteoric water and insects appear to be the most important agents, under Wisconsin conditions, for disseminating the secondary inoculum. In some instances, meteoric water seems to be an even more important agent than insects in the spread of the secondary inoculum for both blossom blight and twig blight.

Laboratory and greenhouse studies of the mode of entry of *Bacillus amylovorus* indicate that, under favorable conditions, the fire blight pathogene can gain access to the tissues of the host through stomata. Cases of stomatal penetration have been found in inoculated tissues taken from (1) young apple leaves, (2) sepals of unopened apple blossoms, and (3) the inside of the receptacle cups of apple and pear flowers that were open when inoculated. In each case the inoculation was made by atomizing with a pure suspension of *B. amylovorus* in water.

Studies of the migration of *Bacillus amylovorus* suggest that in the initial stages of invasion of the host tissues the fire-blight bacteria are free-swimming in the liquid in the intercellular spaces. In later stages of infection they were found embedded in a substance

¹¹ Since this manuscript was submitted Dr. E. C. Tullis has published certain studies on the overwintering and modes of infection of fire blight. (Mich. Agr. Expt. Sta. Tech. Bul. 97, 32 p. 1929.) In the main Doctor Tullis's conclusions are similar to those of the author.

which stains differently than do the bacteria. The nature of this substance was not definitely determined. However, conclusive evidence that such masses are true zoogloea seems to be lacking. In the advanced stages of the disease, *B. amylovorus* was found within the cells. In some instances the bacteria appeared to have migrated from one cell to another through apertures in the walls.

Studies of the parasitism of *Bacillus amylovorus* suggest that the host cells are probably killed by plasmolysis following the extraction of water from the cell contents. Toxin production was not demonstrated with the methods used. In a number of hold-over cankers which were examined histologically, the bacteria were found within the sieve tubes of the phloem in the area immediately surrounding the discolored margin of the canker. In several instances the bacteria were found so crowded in the sieve tube cells of the phloem that individual bacterial rods were distinguishable only with difficulty. No evidence of cyst formation was found.

All varieties of apples grown in Wisconsin are susceptible in a greater or less degree to fire blight. However, such varieties as the Transcendent, Yellow Transparent, Fameuse, MacMahon, and Wealthy are subject to severe attacks of fire blight, whereas the McIntosh, Dudley, and Northwestern Greening are seldom severely infected.

Preliminary studies of the nature of resistance to fire blight are reported. These studies indicate that resistance is probably due, in part at least, to the laying down of a corky barrier by the host. In some varieties this barrier seems to be laid down very quickly after infection.

Studies of variability in varietal resistance to fire blight are reported. Variations in temperature within the limited range used did not appear to have any significant influence on the expression of resistance to fire blight. Subjecting so-called resistant varieties to variations in moisture, however, did seem to affect resistance.

The results of studies of the influence of pollination on the susceptibility of flowers to infection by *Bacillus amylovorus* were not fully conclusive. They show, however, that apple flowers may remain susceptible to infection by *B. amylovorus* for at least 150 hours after pollination.

Control experiments conducted during 1926, 1927, and 1928 are reported. The value of the results is limited because of the small amount of disease which developed in these years. However, excision of blight lesions in the dormant period appeared to cause some reduction in the amount of blossom and twig blight in the following season. The control of aphids did not result in any significant diminution in the amount of blight. It should be borne in mind, however, that comparatively little twig blight occurred under the conditions of these experiments. Bordeaux mixture, 3-6-50, applied at blossoming time afforded little protection from blossom blight of apples in 1926 and 1927. Experiments are reported in which an effort has been made to discover a suitable bactericidal treatment which would inactivate such inoculum as escapes the excision process. Thus far, the results from such studies are not conclusive.

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STUDIES OF QUALITY AND MATURITY OF APPLES¹

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INTRODUCTION

The term maturity as used in discussing crop growth and harvest is indefinite in meaning. It does not define or locate an exact condition or date before which the material under discussion is immature, or at which it is mature, or after which it is overmature. Apples harvested extremely early are commonly of poor quality for eating, and if harvest is delayed until the crop is falling it is usually of poor storage quality. These facts form the basis of the problem discussed in this paper.

REVIEW OF LITERATURE

Magness and Diehl (*13*)² emphasize the fact that the ripening process in apples is a combination of many factors. The different changes that take place in the fruit as it approaches maturity on the tree may continue at varying rates in different seasons and under different climatic and nutritional conditions. Magness and Diehl (*12*) state that the general changes in apples while ripening on the tree are (1) changes in acidity, (2) changes in sugar and starch content, (3) changes in the pectin content, and (4) changes in the moisture content. Plagge, Mancy, and Gerhardt (*17*) are of the opinion that some of the changes associated with the ripening of apples on the tree and in storage are a loss of moisture, acidity, dextrins, starch, and acid-hydrolyzable material. They found an increase in specific gravity, sugars, and soluble pectin.

Browne (*3*) states that the sweetness of an apple is dependent on the absence of malic acid rather than on an excess of sugar as is commonly supposed. Apples frequently contain more sugar than fruit of a sweeter kind. Magness and Burroughs (*11*) state that acidity is an important factor in the flavor of fruit and is largely responsible for its freshness of taste. Magness (*10*) finds that sugars and acids are intimately linked with fruit flavor. Magness and Diehl (*12*) believe that acidity is of much importance in determining the quality of fruit.

Bigelow, Gore, and Howard (*1*) recognize that the variation in composition of fruit on the tree introduces a possible variation that is exceedingly difficult to calculate. They feel that only by obtaining a large amount of evidence can a clear indication be obtained of the changes that occur. Jones and Colver (*8*) believe that the composition of apples may vary with their position on the tree. Magness

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² Reference is made by number (*italic*) to "Literature cited," p. 638.

and Diehl (13) note a wide variation in sugar content in apples of the same variety and from the same trees grown in Virginia during the two seasons 1923 and 1924. The 1924 crop was distinctly lower in sugar content.

Total sugars have been shown to increase through part or all of the growing season (7, 18), as well as during storage (12, 13). It is believed that an important change is that of starch to sugar. Probably certain pectin and hemicellulose compounds are converted into sugars (11). Starch has been shown to decrease during the growing and storage season (1, 12) and invert sugar has been found to increase during growth and storage (1, 19, 15). According to Thompson and Whittier (19), the reducing material consists mainly of levulose. Sucrose has been shown to increase during ripening and to decrease in storage (15, 17). Magness and Burroughs (11) find that sugar changes are much less affected by storage conditions than are acidity changes.

A decrease in acidity during growth has been noted by several workers (1, 3, 17, 18). Magness and Diehl (12) suggest that the most accurate conception of acidity changes in apples while on the tree is that of high acid content early in the season followed by a dilution as the fruit increases in size with a consequent low acid concentration in the mature fruit. Because of the wide variation in acidity in individual apples it would be necessary to use large samples to determine accurately what influences the rate of acidity loss.

Magness and Burroughs (11) find that in stored apples the acidity varies greatly with the conditions under which the fruit is stored, and Magness and Diehl (13) call attention to the fact that because of the wide variations existing particularly in the acid content of different individual apples, it is difficult to determine accurately the decrease in acidity under different storage conditions. Haynes's (6) acidity determinations on individual apples show that the average deviation from the mean was 6 to 15 per cent of the mean. Other determinations showed the percentage to vary from 13 to 19. Evans (5) finds a rather wide range in acidity and total sugars in samples of identical history and like degree of maturity.

Bigelow, Gore, and Howard (1) note that the chemical composition of apples changes very rapidly after they are picked from the trees. In six days the composition of summer apples changed from 24 per cent starch, 4.6 per cent sucrose, and 24 per cent invert sugar to 5.9 per cent starch, 12.9 per cent of sucrose, and 38.2 per cent sugar. This was a greater change in starch than took place in apples which remained on the tree for 43 days. Plagge, Maney, and Gerhardt (17) think the time of picking or condition of maturity at the time of storage within certain limits affected but little the ultimate chemical composition of the apples in cold storage. Under cold-storage conditions acidity, dextrin, starches, and acid-hydrolyzable material gradually decreased with a correspondingly slow increase in simple and disaccharide sugars.

MATERIALS AND METHODS

Jonathan apples from the experiment station orchard at Pullman, Wash., formed the basis of the larger part of the work reported in this

paper. Work was also done on Rome Beauty, Winesap, and Delicious from Yakima and on Jonathan and Delicious from Clarkston, Wash.

The data for 1923 and 1924 crops include measurements and analyses on samples from a period several weeks before the ordinary harvest date through harvest and through the ordinary period of storage. These samples were collected at intervals representing as nearly as possible definite stages in the maturity of the fruit. The fruits were hand picked from all parts of the trees and brought directly to the laboratory, where some were used by the horticultural division for the study of physical characteristics, including pressure tests as described by Morris (14), and for the recording of the histological data. The remainder were used for chemical analysis.

During the 1924 season, duplicate samples were collected to obtain data on the natural variation occurring between samples from the same tree or from the same orchard. Duplicate determinations were then made on each of the duplicate samples.

Six to eight representative apples were quartered and cored and a thin slice was taken from each of the various quarters until the required weight of sample was obtained.

To determine the dry weight 50 gm. of the sample were weighed into a tared beaker, evaporated down on the steam bath for a short time, placed in a vacuum oven at 70° C. and 15 cm. pressure for five hours or to constant weight.

Ash was determined on the sample remaining from the moisture determination by burning in a muffle furnace.

To determine the sugars and acid-hydrolyzable reducing material, 25-gm. samples were covered with 100 c. c. of 95 per cent alcohol, the acid was neutralized, and the sample boiled vigorously for a few minutes on the steam bath. The alcohol was carefully decanted and the residue extracted with alcohol for 12 hours. The extract was added to the original filtrate and made up to 500 c. c. with distilled water. Reducing substances were determined on 10 c. c. of this solution by the Munson and Walker method, weighing the cuprous oxide. Fifty cubic centimeters of the above solution were hydrolyzed by adding 10 c. c. of hydrochloric acid, making up to 100 c. c., and allowing to stand overnight. Reducing substances were again determined and calculated as dextrose. The residue from the alcohol extract was dried and hydrolyzed with 125 c. c. 2.5 per cent hydrochloric acid under a reflux condenser for two and a half hours. The solution was cooled and nearly neutralized with sodium hydroxide. This was made up to 250 c. c. and reducing substances were determined on a 10 c. c. aliquot. This is referred to as alcohol-insoluble acid-hydrolyzable material.

For the determination of acid 50 gm. of the sample were placed in a beaker, 150 c. c. of distilled water were added, and the material immediately boiled. The solution was made up to 500 c. c. and allowed to stand with frequent shaking for three days, 2 c. c. of toluol having been added to prevent decomposition. One hundred cubic centimeters of this solution were then titrated with 0.1 N sodium hydroxide. The end point was determined by phenolphthalein as indicator.

A portion of the sample was ground through a hand mill and the juice expressed. The viscosity was determined at 25° C. Hydrogen-ion concentration was determined with a Hildebrand electrode, using a Leeds and Northrup type K potentiometer.

The acidity of the juice was determined by titrating 25 c. c. of the juice with 0.1 N sodium hydroxide, the end point being determined electrometrically. Titration curves were constructed.

Nitrogen was determined on 25 gm. of the sample by the ordinary Kjeldahl method.

RESULTS IN 1923

During the 1923 season Jonathan apples were collected from the station orchard on seven different dates extending from August 30, when the fruit was still decidedly green, to October 23, which was well past the commercial harvest date. The results obtained from the chemical analyses of these samples are presented in Table 1. The data are the averages of determinations on duplicate samples taken from individual orchard samples.

TABLE 1.—Composition of Jonathan apples harvested on the Washington Experiment Station farm in 1923

Date harvested	Dry matter		Ash	Acid as malic		Nitrogen	Sugar			Alcohol-insoluble acid-hydrolyzable material	Remarks
	P. ct.	P. ct.		P. ct.	P. ct.		Reducing	Sucrose	Total		
Aug. 30	-----	0.280	0.91	0.060	8.77	1.87	10.64	3.72			Very green, very hard, 5 per cent red, sour, no aromatic flavor, poor eating quality.
Sept. 10	-----	.269	.97	.065	8.40	2.14	10.54	3.66			Green, very hard, 8 per cent red, no aromatic flavor, poor eating quality.
Sept. 20	18.84	.345	.84	.058	8.50	2.72	11.22	3.72			Green, very hard, 20 per cent red, slight aromatic flavor, poor to medium eating quality.
Sept. 26	19.23	.286	.96	.050	8.89	3.25	12.14	3.67			Green, hard, 50 per cent red, distinct aromatic flavor, medium eating quality.
Oct. 9	17.18	.208	.83	.046	7.50	3.94	11.44	2.02			Green to yellowish green, hard, 75 per cent red, distinct aromatic flavor, good eating quality.
Oct. 16	19.26	.262	.78	.047	8.98	3.54	12.52	2.07			Greenish yellow, hard, 85 per cent red, high aromatic flavor, very good to best eating quality.
Oct. 23	17.97	.234	.73	.053	8.98	4.74	13.72	1.38			Greenish yellow, firm to hard, 90 per cent red, medium to high aromatic flavor, very good to best eating quality.

The data do not indicate any consistent or progressive variation in the percentage of dry matter through the growing season. The same is true of the ash and nitrogen, although it may be said that there seems to be a tendency for the nitrogen to decrease. The percentage of acid may be interpreted as showing a tendency to decrease as the apple matures, although in the light of the average variation in Table 5 this decrease is not significant since the actual amount of acid per apple increases slightly. The total sugars increased about 3 per cent from August 30 to October 23. This increase is almost entirely accounted for by the increase in sucrose, since the change in the amount of reducing sugars was small. Sucrose showed a decided increase during the 1923 season; on October 9 there had been an increase of about 110 per cent over the amount found on August 30. Two weeks later, on October 23, the total increase was over 150 per cent.

The question arises as to the source of this additional sucrose. In this connection it is interesting to study the variation in the

percentage of alcohol-insoluble acid-hydrolyzable materials (called starchy material and starch in Washington Bulletin 205 (16)). This portion of the apple undoubtedly includes pentosans, cellulose, hemicellulose, glucosides, pectoses, and other carbohydrate bodies besides starch that undergo hydrolysis and are converted into reducing substances on boiling with hydrochloric acid but can not be considered starch, as emphasized by Magness (9). These alcohol-insoluble acid-hydrolyzable materials decreased from about 3.7 to about 1.4 per cent, or a decrease of about 63 per cent.³ During the same period the sucrose increased from 1.87 to 4.74 per cent, or an increase of more than 150 per cent. The weight of alcohol-insoluble acid-hydrolyzable material per apple increased up to September 26, after which there was a drop in the amount of this fraction. The weight of sucrose per apple steadily increased.

Three possible sources of sucrose are suggested. A part may come from the alcohol-insoluble acid-hydrolyzable portion, since this shows a percentage decrease and also a decrease in the amount per apple after a certain date. It is also possible that increased enzymatic activity may have converted a part of the alcohol-insoluble material, which is not hydrolyzable by hydrochloric acid, into sucrose. The principle part is, however, probably synthesized from hexoses, which are translocated from the leaves.

The percentage of the alcohol-insoluble acid-hydrolyzable fraction does not show a progressive decrease throughout the period of this study, but remains practically constant through September 26, and then drops very decidedly by the next picking date. There were no killing frosts during this interval. On the other hand, the quantity of sucrose shows a fairly regular progressive increase.

PHYSICAL-CHEMICAL DATA

In addition to the chemical data just discussed, certain physical-chemical measurements have been recorded and are presented in Table 2. These were made on the juice expressed from the apples. The results of the measurement of conductivity on the same water extract which was prepared for the determination of total acidity are also given.

TABLE 2.—Physical constants of juice from Jonathan apples harvested in 1923

Date harvested	Specific gravity	Viscosity	pH	Acidity (per cent)	Conductivity	Conductivity of water extract
Aug. 30	1.0539	1.76	2.84	1.02	39×10 ⁻⁴	5.76×10 ⁻⁴
Sept. 10	1.0514	1.71	2.77	.96	36.8×10 ⁻⁴	6.22×10 ⁻⁴
Sept. 20	1.0602	2.07	1.32	1.10	31.1×10 ⁻⁴	6.22×10 ⁻⁴
Sept. 26	1.0458	1.75	3.26	.75	31.8×10 ⁻⁴	8.19×10 ⁻⁴
Oct. 9	1.0621	1.89	3.13	.88	31.8×10 ⁻⁴	5.68×10 ⁻⁴
Oct. 16	1.0616	1.84	3.25	.73	29.5×10 ⁻⁴	5.91×10 ⁻⁴
Oct. 23	1.0613	1.78	3.35	.64	39.5×10 ⁻⁴	5.88×10 ⁻⁴

³ In order more clearly to indicate the comparison with the sucrose increase of 150 per cent, it may be stated that these alcohol-insoluble acid-hydrolyzable materials were 170 per cent higher at the beginning than at the close of the period.

The measurements in these data which suggest a progressive variation are those of hydrogen-ion activity and acidity of the juice as measured by electrometric titration. There is a greater variation in the acidity of the juice than in the total acidity as determined on the water extract, and varying in the same direction is the hydrogen-ion activity, which is decreasing (increasing pH).

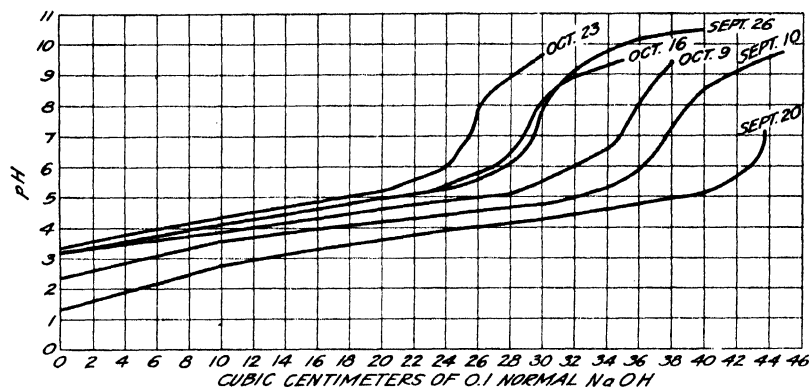


FIGURE 1.—Titration curves showing acidity of juice of apples harvested in 1923 on dates indicated

The titration curves for the juice are presented in Figure 1. These indicate graphically the variations in pH and in total acidity mentioned above, and in addition they show that the buffer value of the juice is about the same at the different stages of maturity.

TABLE 3.—Changes in composition of Jonathan apples harvested October 5, 1923, and stored at 45° to 50° F.

Date	Dry matter	Ash	Acid as malic	Nitrogen	Sugar			Alcohol-insoluble acid-hydrolyzable material
					Reducing	Sucrose	Total	
Dec. 5	Per cent 15.74	Per cent 0.31	Per cent 0.59	Per cent 0.050	Per cent 7.98	Per cent 3.44	Per cent 11.42	Per cent 1.07
Dec. 7	17.19	.232		.052	8.70	3.86	12.56	.89
Jan. 3	17.10	.11		.046	8.94	3.66	12.60	.79

STORAGE RESULTS

The data obtained on the Jonathan apples from the station farm which were placed in storage are presented in Table 3. These apples were harvested and stored at 45° to 50° F. on October 5.

Apples which varied in size were selected in an effort to determine whether the composition varied with size and also whether size were correlated with keeping quality, for Morris (14) had found that large fruits of the same variety, from the same tree, and harvested at the same time, do not keep as well as smaller fruits. The data in Table 3 do not suggest such variation. A comparison of these data with those for the fruit picked from the trees (Table 1) shows that a further decrease of acid and of the alcohol-insoluble acid-hydrolyzable material takes place after the fruit is placed in storage.

RESULTS IN 1924*

In 1924 the work was enlarged to include the analysis of duplicate samples. As previously stated, duplicate determinations were made on all samples analyzed in 1923. In 1924 a sample was collected from a section of the orchard to be studied, care being exercised to make the sample as representative as possible from the horticultural and chemical standpoint. The same trees were sampled a second, and, in several cases, a third time. Duplicate determinations of each of the fractions studied were made on each of the duplicate or triplicate samples.

The data obtained on the samples collected during the growing season of 1924 are presented in Table 4 and Figure 2.

The figures in Table 4 represent the average of the duplicate and triplicate determinations. Comparing the variation between the amount of dry matter in duplicate and triplicate samples shown in Table 4 and that for storage apples shown in Table 6, it was found

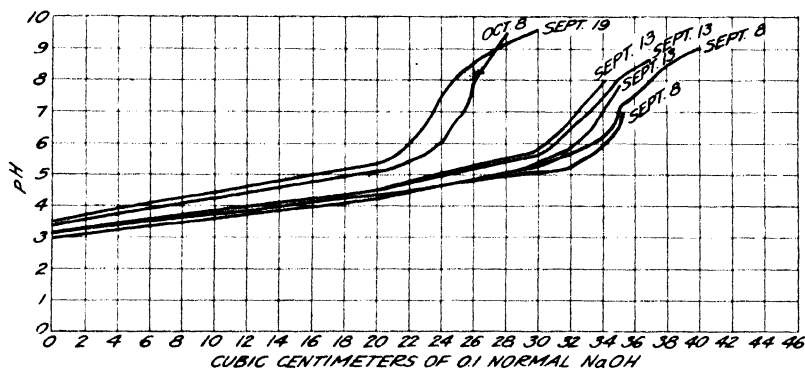


FIGURE 2.—Titration curves showing acidity of juice of apples harvested in 1924 on dates indicated

that the average variation between the duplicate or triplicate samples was 0.684. (Table 5.) The average variations for other determinations are also shown in Table 5.

The data for the 1924 storage apples are given in Table 6. These apples were harvested and placed in storage at 45° to 50° F. on September 25.

Tables 4 and 6 also give the averages of the analyses of duplicate samples. If we assume that a variation three times the average variation is a significant variation it would appear that the variation in dry matter is not significant during the growing season, since the variation between the earliest and latest picking is only 0.82 per cent and no intermediate variation is equal to more than twice the average variation. Viewed in the same way it is doubtful if there is a significant variation in dry matter during storage.

Considering the total acid, it again appears that the largest variation is less than twice the average variation. The data for the amount of acid in storage might be interpreted by inspection to show a decrease. However, it is only twice the average variation.

* The writers desire to express their thanks to Tom Beal for assistance in the analytical work of 1924.

TABLE 4.—Composition of duplicate and triplicate samples of Jonathan apples harvested on the Washington experiment station farm in 1924

Date of harvest	Dry matter	Ash	Acid as malic	Acidity of juice	Nitrogen	Sugar			Alcohol-insoluble acid-hydrolyzable material	Remarks
						Reducing	Sucrose	Total		
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
Sept. 1.....	{ 17.40 17.30	{ 0.278 .327	{ 0.808 .912	-----	{ 0.0825 .058	{ 7.19 6.85	{ 1.87 1.98	{ 9.06 8.83	{ 3.62 3.70	Very green, very hard, 5 per cent red, no aroma, very poor eating quality.
Average.	17.35	.303	.890	-----	.0603	7.02	1.93	8.95	3.66	
Sept. 8.....	{ 17.39 *18.47 *18.56	{ .312 .255 .222	{ .877 .854 .820	{ 0.819 .765 .760	{ .050 .047 .046	{ 6.52 7.15 6.84	{ 3.46 3.37 2.79	{ 9.98 10.52 9.63	{ 3.35 3.53 3.40	Very green, very hard, 12 per cent red, very slight aromatic flavor, very sour, low to medium eating quality.
Average.	18.14	.263	.850	-----	.051	6.84	3.21	10.04	3.43	
Sept. 13.....	{ 16.85 *16.78 17.27	{ .322 .290 .319	{ .872 .850 .861	{ .856 .785 .800	{ .052 .050 .045	{ 6.27 6.94 6.53	{ 2.89 2.49 2.39	{ 9.14 9.43 8.92	{ 2.16 2.40 2.15	Very green, very hard, 16 per cent red, slight aromatic flavor, poor to medium eating quality.
Average.	16.97	.310	.861	-----	.049	6.58	2.59	9.16	2.24	
Sept. 19.....	{ 16.57 15.27 15.82	{ .306 .238	{ .819 .607 .682	-----	{ .058 .040 .040	{ 6.74 ----- -----	{ 1.54 ----- -----	{ 8.28 7.86 7.34	{ 1.76 1.30 1.53	Green, hard, 35 per cent red, slight aromatic flavor, medium eating quality.
Average.	15.89	.272	.703	-----	.046	6.74	1.54	7.83	1.53	
Oct. 8.....	{ 16.10 16.95	{ .223 .226	{ .734 -----	-----	{ .034 .038	{ 7.23 7.54	{ 3.93 3.34	{ 11.16 10.88	{ 1.29 1.29	Yellowish green, hard, 60 per cent red, distinct aromatic flavor, medium to good eating quality.
Average.	16.53	.225	.734	-----	.036	7.39	2.64	11.02	1.29	

* South side of tree.

* North side of tree.

TABLE 5.—Average variations between duplicate samples calculated on 1924 data

Average variation.....	Average variation in—					
	Dry matter	Total acid as malic	Sugar			Alcohol-insoluble acid-hydrolyzable material
			Reducing	Sucrose	Total	
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Average variation.....	0.684	0.083	0.346	0.478	0.571	0.159

TABLE 6.—Changes in composition of duplicate and triplicate samples of Jonathan apples harvested September 25, 1924, and stored at 45° to 50° F. until dates indicated

Date	Dry matter	Ash	Acid as malic	Nitrogen	Sugar			Alcohol-insoluble acid-hydrolyzable material
					Reducing	Sucrose	Total	
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Oct. 27.....	16.44	0.294	0.586	0.037	7.41	4.71	12.12	0.65
Nov. 4.....	{ 16.57 16.96	{ .307 .238	{ .547 .572	{ .037 .040	{ 7.42 7.48	{ 3.99 4.84	{ 11.41 12.32	{ .75 .71
Average.....	16.77	.273	.560	.038	7.45	4.42	11.87	.73
Dec. 10.....	{ 14.73 16.50	{ .303 .236	{ .500 .552	{ .052 .046	{ 7.41 9.02	{ 3.37 3.18	{ 10.78 12.20	{ .66 .83
Average.....	15.62	.270	.526	.049	8.22	3.27	11.49	.74
Jan. 7.....	15.21	.230	.418	.044	8.54	2.20	10.74	.71

Unfortunately, we do not have a determination of reducing sugar for the samples taken on September 19. The variation from September 1 to 13 is a trifle more than the average variation between duplicate samples. From September 13 to October 8 the reducing sugars increased 0.81 per cent, or more than twice the average variation. In storage on October 27 and November 4 these sugars were practically the same as at the last picking. Over a month later, on December 10, there had been an increase of 0.77 per cent of reducing sugars, which is a trifle over twice the average variation.

The variation between samples in the amount of total sugars is large, as shown by comparison with the average variation. During the growing season the variation is first in one direction and then in the other. On the whole, however, it would appear that there is an increase in total sugars, since the difference between the amount found for October 8 and for each of the previous dates is either practically as great as or greater than three times the average variation for total sugars. It also appears that there was a further slight increase in total sugar for three weeks after the apples were placed in storage. Following this the total sugars appear to have decreased, although this, too, is open to question, due again to the fact that the January 7 analysis was on one sample only.

During growth the changes in sucrose followed in general the changes in total sugars. For the first three weeks in storage there was an increase in sucrose after which there was a decrease, the decrease being greater than that in total sugars. This was due, of course, to the increase in reducing sugars and indicates that sucrose is converted into reducing sugars during storage.

The alcohol-insoluble acid-hydrolyzable material probably shows a greater variation than any of the other fractions determined. This will be further discussed when a comparison of the three years' results is made. This material shows a progressive decrease through the growing period. The total decrease from September 1 to October 8 is nearly fifteen times the average variation. There was a further significant drop in this fraction during the first three weeks of storage, after which it remained practically constant.

COMPARISON OF RESULTS OF 1923, 1924, AND 1925⁵

Considering the data for the three years as a whole, it appears that there is a fluctuation in dry matter but no significant variation. In ash and nitrogen there is likewise little variation. The figures for total acid are probably the most difficult to interpret, but on the basis of the average variation, as discussed above, it appears that there is no significant variation. However, considering the figures, there appears to be a perceptible decrease in total acid, although this decrease is very small in many cases, and in no case is the decrease for the growing season equal to much more than twice the average variation. It would therefore seem best to conclude that there is no definite evidence of a significant decrease as the season advances. The only way in which it can definitely be shown whether or not the acid decreases is to analyze a much larger number of samples. The writers' data agree with those of other workers in showing a perceptible decrease in acid as the fruit ages, but the significance of this

⁵ See reference (16) for 1925 data.

decrease may have been overemphasized. The data for all three years agree in showing an increase in total sugars during the growing season. It is also apparent that this increase is due largely to an increase in sucrose, since there is no progressive decrease in the amount of this material as the season advances.

Of the total sugars it appears that there were appreciably more in the apples in 1923 than in either 1924 or 1925, which showed approximately the same amount. The same is true of the reducing sugars. The amount of sucrose seems to have been about the same for the three years. The amount of alcohol-insoluble acid-hydrolyzable material seems to have been about the same for 1923 and 1924, although the bulk of the decrease in this material came earlier in 1924 than in 1923. This was probably due to the fact that the 1924 harvest season was earlier. The amount of this fraction in the 1925 crop was decidedly less throughout the season than in the other two years, as is readily apparent from Table 7.

TABLE 7.—Percentage of alcohol-insoluble acid-hydrolyzable material in Jonathan apples harvested in 1923, 1924, and 1925

1923		1924		1925	
Date harvested	Per cent	Date harvested	Per cent	Date harvested	Per cent
Aug. 30.....	3.72	Sept. 1.....	3.66	Sept. 2.....	2.13
Sept. 10.....	3.66	Sept. 8.....	3.43	Sept. 9.....	1.49
Sept. 20.....	3.72	Sept. 13.....	2.24	Sept. 16.....	1.17
Sept. 26.....	3.67	Sept. 19.....	1.53	Sept. 23.....	1.40
Oct. 9.....	2.02	Oct. 8.....	1.29	Sept. 30.....	1.13
Oct. 16.....	2.07			Oct. 7.....	.82
Oct. 23.....	1.38				

From these data it would not seem possible to establish a definite level to which this fraction must come in order to indicate a desirable period of maturity, but it is more than probable that it will be necessary to follow the amount of this material through the season to a point where there is a decided drop in the amount. Further work such as was suggested in 1924 is apparently necessary to establish this point.

An interpretation by inspection would suggest that the amount of acid found in the 1925 crop is somewhat less than that in the two preceding crops. This difference is, however, only approximately twice the average variation.

The annual rainfall at Pullman is about 20 to 22 inches. A very small proportion of it falls during the summer. The college orchard is not irrigated, and the soil is usually quite dry during August and September. The apples are small to medium in size, but when harvested late are of good color and of good eating quality. Those of the crop of 1923 were well colored and very small, but continued to increase in size until after the last samples were harvested. The fruits of the 1924 crop were very much larger than those of the 1923 crop, were well colored, and continued to increase in size until after the middle of October.

The season of 1923 was practically without rainfall during August and September. Bright, clear weather prevailed until October 16 with relatively high temperature for the district. August 26 to 31, inclusive, had an average daily maximum temperature of 88° F. and

an average daily sunshine of 12 hours. September had an average daily maximum temperature of 80° and an average daily sunshine of approximately 8 hours. October 1 to 23, inclusive, had an average daily maximum temperature of 57° and an average daily sunshine of 4 hours.

In 1924 there were several light rains during the latter part of August and during September and October. These fell as slow drizzles, and a high atmospheric humidity prevailed during the cloudy days that followed. August 26 to 31 had an average daily maximum temperature of 66° F. and an average daily sunshine of 9½ hours. September had an average daily maximum temperature of 61°, and an average daily sunshine of approximately 8 hours. The first eight days of October had an average daily maximum temperature of 49°, and an average daily sunshine of approximately 4 hours.

The 1923 season was thus characterized by a large amount of sunshine, relatively high temperatures, and little or no rain, while that of 1924 was characterized by light rains, less sunshine, and lower temperatures. As previously suggested, it would therefore be expected that the fruit harvested in 1923 would be of better quality than that obtained in 1924. By reference to Tables 1 and 4 and accompanying legends this is found to be the case, as indicated by the amount of sugars present and by observations on color, flavor, and eating quality.

QUALITY OF APPLES AS RELATED TO GROWTH AND EXPOSURE

Tests of the eating quality of fruit were made of each crop of apples from 1923 to 1926, inclusive. These studies were confined to Jonathan, Rome Beauty, Winesap, and Delicious. A great variation was found in the quality of fruit within a variety and also in the quality of fruit from a single tree. Trees that suffered from drought during the middle and latter part of the summer usually produced fruit of very low quality. This is the first fact made clear in these tests. The similarity of low quality in some fruits from trees that seemed to be well supplied with water to those that were evidently suffering from lack of water, led to a careful study of the effects of environment on tree growth, and of weather on the quality of individual fruits. In this study quality was determined by taste. The fruits were graded as excellent, good, medium, and poor according to the ratings for quality given by 109 persons who tasted the fruits. In 1925 and 1926 the quality of large numbers of fruits growing under the environmental conditions indicated in Table 10 was determined at harvest and after 30 and 90 days of storage.

Dasture (4) in a study of the relation of water content to photosynthesis found that a close relationship exists between the rate of assimilation of food by the plant and the water content of the foliage.

Many tests to determine the loss of water by the fruit were carried out. The results of one of these made in late August are presented here. Twenty fruiting spurs with fruit attached were removed from the tree, taken to the laboratory, and weighed before 8 a. m. Ten of these were left with the fruit and foliage attached. The fruit of the other 10 was separated from the twig. The lots were weighed and left on tables in the laboratory fully exposed to the dry air at a temperature of 78° F. The results are given in Table 8.

TABLE 8.—*Loss of water by apple fruit and foliage when left exposed to dry air on a laboratory table at 78° F.*

Item	Average weight at—		Weight loss
	8 a. m.	5 p. m.	
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
Apples with twig and foliage attached.....	159.2	152.1	7.1
Apples with twig and foliage detached.....	145.5	145.1	.4
Twigs and foliage only of second lot above.....	11.2	7.0	4.2

Apples attached to the twigs carrying the foliage became very much wilted and showed shriveling of the skin while the detached fruit remained apparently plump and was only slightly wilted. Fruits attached to the largest amount of foliage lost weight most rapidly, while those attached to a few leaves only lost weight more slowly and the foliage remained green for a longer time.

Apples on trees carrying a heavy load of foliage and a medium to light crop of fruit are often noticeably wilted during the very hot weather of summer. This is most apparent on fruit attached to twigs carrying a large amount of foliage and on varieties that have very large leaves. Water evaporates from the foliage more rapidly than it can be supplied by the tree and the twig draws some direct from the apples.

Brooks and Fisher (2) showed that water-cored apples and the adjacent foliage had a high concentration of sap and that this sap had a very high osmotic pressure. They also showed that fruit in the part of the tree most exposed to sun was most water cored. Artificial shading seemed to prevent water-core development.

Von Ende (15) states that a delicate balance exists between the enzyme processes which take place in the carbohydrate metabolism, that the process is reversible, and that the synthesis of starch and sucrose is a matter of the concentration of the hexoses. Sucrose is formed when the hexoses reach a certain concentration and starch is formed when the sucrose reaches a certain concentration.

Temperature readings of the foliage are presented in Table 9. The temperature of the leaf does not indicate perfectly the rate of transpiration, but the two are closely related. Each temperature given in the table represents an average of 60 to 130 determinations. These readings, taken in an orchard at Clarkston, Wash., with a thermocouple and galvanometer, indicate clearly that in the early part of the forenoon the foliage of the different lots gave off moisture at approximately the same rate. At midday the foliage attached to the spurs carrying fruit gave off moisture more rapidly than that attached to spurs of the same length without fruit. Evaporation of water from the foliage on twigs attached to spurs carrying fruit showed a slowing down very early in the day and continued at a slower rate than that on spurs without fruit until late in the day.

In Table 10 are shown the records for quality in Jonathan apples growing on spurs of different lengths in exposed and in shaded situations. These data represent quality as determined by taste only and have no reference to the appearance of the fruit as to color, size, or hardness. Each lot contained 100 apples. The fruit was about

average in range of hardness for the date of harvest and was from trees growing in a clean-tilled orchard. Fruit from an adjacent orchard carrying an alfalfa cover crop ranged practically the same in the different grades of quality.

TABLE 9.—*Leaf temperatures of Jonathan apple trees in orchard at Clarkston, Wash., September 12, 1925*

Lot No.	Type of spur	9 a. m.	11 a. m.	12.30 p. m.	2 p. m.	4 p. m.	5 p. m.
	Air temperature in degrees Fahrenheit.....	70	81.0	85.0	89.0	82.0	75.0
1	Short nonfruiting.....	67	74.0	78.0	83.0	77.0	72.5
2	Short fruiting.....	67	73.6	77.5	83.7	76.0	71.7
3	Long nonfruiting.....	67	77.0	83.2	88.0	81.1	74.2
4	Long fruiting.....	67	75.1	81.5	87.0	80.0	73.0
5	Medium length nonfruiting with twig growth.....	67	74.0	82.0	86.7	80.1	74.0
6	Medium length fruiting with twig growth.....	67	74.0	81.0	85.1	79.8	73.4

TABLE 10.—*Quality of Jonathan apples harvested in Clarkston, Wash., September 10, 1926, as related to exposure to the sun*

[Tested Oct. 22, 1926]

FRUIT AND FOLIAGE EXPOSED TO SUN

Lot No.	Type of spur	Percentage of fruit grading—			
		Excel- lent	Good	Me- dium	Poor
1	Fruits attached to twigs or spurs over 18 inches long, without twig growth.....	0	3	9	88
2	Fruits attached to spurs 1 to 4 inches long, without twig growth.....	10	70	20	0
3	Fruits attached to spurs 8 to 12 inches long, without twig growth.....	8	32	51	9
4	Fruits attached to spurs 4 to 10 inches long, carrying short twig growth.....	41	42	12	5

FRUIT AND FOLIAGE PARTIALLY OR ENTIRELY SHADED

1	Fruits attached to twigs or spurs over 18 inches long, without twig growth.....	0	0	7	93
2	Fruits attached to spurs 1 to 4 inches long, without twig growth.....	0	7.5	41	51.5
3	Fruits attached to spurs 8 to 12 inches long, without twig growth.....	0	7	29	64
4	Fruits attached to spurs 4 to 10 inches long, carrying short twig growth.....	0	5	43	52

The fruits of highest quality were those well exposed to the sun and attached to relatively short, vigorous spurs on large branches or on longer spurs of sufficient vigor to produce a fruit and a twig at the same time. Fruit produced on long, slender twigs or spurs with few leaves and that grown on the long spurs, as lot 4 of Table 9, was of distinctly inferior quality.

The highest quality or highest flavored fruit was practically always found on the spurs which produced from the same winter bud a short, leafy twig, carrying 10 or more leaves, as lot 6, Table 9 and lot 4, Table 10, in contradistinction to those which produced the normal number of 5 to 7 leaves. It was noted also that maximum quality was quite uniformly found to exist in such fruit after three or four successive days of clear, dry weather, and that the lower quality of fruit was harvested from the same trees after a period of cloudy or rainy weather with lower temperatures.

High quality in apples seems to be closely related to medium growth and exposure to strong sunshine and dry air. Extreme drying of the foliage and fruit prevents functioning and injures quality. A constant high-moisture content of the plant provides for such perfect assimilation of the plant foods that there is only a medium or normal quantity stored in the fruit. The superior quality is developed under conditions between these extremes, that is, under such conditions as allow the maximum amount of sugars and other materials that improve flavor to be stored in the maturing fruit. This is probably one cause of the difference in quality of fruit from different districts, and also in different crops from the same orchard.

In two instances in 1924, duplicate samples of Jonathan apples were taken from the north and the south sides of the same tree and analyses were made. The fruit and adjacent foliage on the south side of the tree were exposed to the direct rays of the sun three to five hours longer each day than that on the north side, and the fruit showed more red color. No other recognizable difference in environment was apparent when the samples were gathered. These data are included in Table 4 under the dates of September 8 and 13. In both instances the total sugars, reducing sugars, alcohol-insoluble acid-hydrolyzable material, and total carbohydrates were higher in the samples taken from the south side. In one instance the sucrose was decidedly higher, while in the other it was a little lower in the sample from the south side. In both instances the insoluble material was lower in the samples taken from the south side of the tree.

Samples rating Fancy and C grade, 50 and 20 per cent, respectively, good red color, were taken on February 22 from a storage lot of Jonathan and one of Rome Beauty apples, and analysis made, with the results shown in Table 11. These results show that the sugars are higher in the Fancy than in the C grade apples. The difference in the amount of sucrose found in the two grades is rather striking. The percentage of acid, however, is practically the same. From these data it would therefore appear that for dessert quality in apples the quantity of sucrose present is more important than the quantity of acid.

TABLE 11.—*Composition of Fancy and C grade Jonathan and Rome Beauty apples taken from storage February 22*

Variety and grade of apple	Dry matter	Ash	Acid	Sugar		
				Reducing	Sucrose	Total
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Jonathan, fancy.....	15.96	0.256	0.45	8.98	2.32	11.30
Jonathan, C grade.....	15.24	.263	.41	8.54	1.21	10.15
Rome beauty, fancy.....	15.38	.185	.35	8.09	2.01	10.10
Rome beauty, C grade.....	12.44	.167	.27	6.63	1.32	8.25

Highest quality of the Jonathan, or the highest flavor of fruit, is found only in the highest colored fruits but is not uniform in proportion to red color. This has been found quite uniformly true of the fruit of the well-grown commercial crop. The fruits of medium size are usually of the best quality. This applies to the fruit that matures early, in midseason, or late on the tree. Time of harvest of

the material has apparently a direct relationship to quality in so far as it has a direct relationship to color attained by the fruit at the time of harvest.

The excellency of taste of apples bears the same relationship to color in fruit that is ripened or reaches its maximum dessert quality in storage as it does to that which reaches its maximum quality while on the trees. In the lower altitude, longer-growing-season districts of Washington, Jonathans are ripened and become of excellent quality on the tree, although the fruit may be removed from the tree, placed in storage, and have good storage life if harvested before the tissues have softened. The material grown in the higher altitude, shorter-season districts in some years will not reach the point of ripening on the trees, but must be harvested to avoid freezing before maximum quality or even full growth has been attained.

What is said in regard to color of the Jonathan apple applies equally to the Delicious, Winesap, and Rome Beauty. The Delicious showed the same variation as the Jonathan, although the formation and spur characteristics of the Delicious are quite different from those of the Jonathan.

In 1926, the Delicious crop on selected trees at Clarkston developed water-cored fruits before late harvest. The trees that were making poor growth, with very short twigs and relatively light foliage, developed fruit of medium size, high color, and medium to good quality with less than 5 per cent water core. Forty per cent of the fruits produced on trees making a medium growth, and with the crop thinned to a medium heavy load, were water-cored.

Water-cored fruit was more common on some classes of spurs than on others. On short spurs attached to large branches 10 per cent of the crop was water-cored; on long spurs attached to large branches 36 per cent was water-cored; while on medium-length spurs that had produced both fruit and twigs from the same winter bud, 70 per cent was water cored.

This distribution of water-cored material is more definite and clear-cut than any classification that could be made of the eating quality of the fruit attached to different twigs.

SUMMARY

The authors are unable to present at this time a system of measuring or defining the quality of apples that is accurate and practical for both the orchardist and the chemist.

Data on the composition and quality of Jonathan apples collected during several seasons are presented. A variation in duplicate samples from the same tree is noted, and this must be taken into consideration in interpreting results. Grade should be considered in taking samples, and also probably the position of the fruit on the tree.

The level of sugars and other fractions varies in different years. The dry matter, ash, nitrogen, and reducing sugars show no progressive variation during the growing season. The total sugars increase, due to an increase in sucrose.

The percentage of acid present shows a tendency to decrease during the growing season, but in view of the variations noted, it is doubtful whether much significance can be attached to the small change in

the quantity of acid. For dessert quality in apples the amount of sugar present seems to be of more importance than the amount of acid.

The decrease in the percentage of the alcohol-insoluble acid-hydrolyzable fraction during the growing season is more striking than the change in any other fraction. The constituents of this fraction should be studied further.

Such climatic factors as temperature, sunshine, and rainfall have a decided effect upon quality of fruit and the variation of quality from year to year. The taste of the apple as judged by different individuals varies, but it seems to be the most dependable basis which we have and the one upon which the trade must rely.

The best quality of apple is developed when the fruit and adjacent foliage are well exposed to sunshine and dry air. Conversely, shading the fruit and its adjacent foliage tends to retard quality development.

There is great variation in the quality of fruit produced by the trees in most of the orchards from which samples were taken, and the quality varies most in the trees carrying extremely dense foliage or casting heavy shade.

The method of pruning practiced in the apple orchards of Washington has a great influence over the quality and uniformity of the quality of the crop of apples produced.

Apples that reach full growth on the tree attain maximum quality as compared to those that are harvested before full size is attained.

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FURTHER STUDIES OF COTTON ROOT ROT IN ARIZONA, WITH A DESCRIPTION OF A SCLEROTIUM STAGE OF THE FUNGUS¹

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INTRODUCTION

During recent years investigators have obtained conclusive evidence that the destructive plant disease of the Southwestern States, often called Texas root rot, is caused by the fungus *Phymatotrichum omnivorum* (Shear) Duggar (3).²

Although much study has been given to this disease, little information is available in regard to the life history of the fungus, its physiology, or the factors that effect its distribution. Recent studies have added much to the general knowledge of the habits of the fungus and its ecology, but many important questions remain unanswered.

In previous publications the writers (6, 7, 8, 9) have reported the results of experiments which indicated that the disease could be controlled to a limited degree in cotton and alfalfa fields by the use of organic manures, trench barriers, and soil disinfectants. The behavior of the fungus in artificial cultures containing different kinds of plant roots and the partial success obtained in efforts to inoculate healthy plants with pure cultures were also reported. Since the fact was established that the fungus grew readily on dead plant tissues in cultures and that active mycelium on decayed roots and stumps of native plants gave rise to root-rot infection in cultivated crops when planted in virgin soils, it was suggested that the fungus might be capable of existing in the soil as a saprophyte.

During the season of 1928 additional information was obtained which appears to be of sufficient importance to justify a further report. The investigations included a study of the types of food on which the fungus may subsist, the manner in which it spreads through the soil, and the depth at which it may function. Further inoculation and control experiments were conducted, and a better knowledge of some of the phases of its life history was obtained. The discovery of what appears to be a true sclerotial development of the fungus in soil cultures seems to be of special importance in the bearing it may have on the effectiveness of control measures, such as rotation of crops, fallowing, flooding, and chemical treatments. The results of these investigations and observations are reported in this paper.

CULTURES OF THE ROOT-ROT FUNGUS ON DEAD ROOTS

Although there is ample proof of the pathogenic character of the fungus *Phymatotrichum omnivorum*, there appears to be a lack of

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² Reference is made by number (italics) to "Literature cited," p. 675.

agreement among investigators as to whether it is a facultative parasite as originally described by Shear (15) or an obligate parasite which can not long survive in the soil independent of living hosts. Taubenhauß and Killough (16) state that "the causal organism in nature dies out with the death of its host" and that it "is unable to maintain itself on dead organic matter or in the soil." On the other hand, Peltier, King, and Samson (13), Ratliffe (14), and King and Loomis (9) have



FIGURE 1.—Effect of inoculations with pure cultures of the cotton root-rot fungus on Acala cotton plants grown in tanks of disease-free soil. The plant on the left was inoculated on August 9 with the contents of a quart fruit jar containing a pure culture of the fungus on dead tissues of cotton plants in alternate layers with sand. The inoculated plant wilted on August 14, and the disease was then communicated through the soil to the other plant, which wilted on August 26.

reported observations wherein roots that had been dead for a long period were covered with root-rot mycelium which was the source of conidial mats, in some cases producing centers of infection.

Since it was apparent that the effectiveness of any cultural methods of control would be likely to be influenced by the question whether the fungus was able to maintain itself in the soil independent of a

living host, it seemed important to the writers to endeavor to establish final proof. A large number of pure cultures were prepared by inserting bits of dissected pseudosclerotia from a type culture into quart fruit jars containing alternate layers of sand and dead roots and stems of cotton plants. To eliminate the possibility of extraneous fresh-plant tissues being present in the culture jars, the sand used was thoroughly sifted and heated to a high temperature before final sterilization. Only such plant tissues were selected as had reached an advanced stage of decomposition, having been cut into sections and buried in the soil for one or two years.

When the cultures had grown until all of the root and stem tissues were covered with the mycelium and large strands had reached the bottom of the jars, the contents were removed and inserted in a hole 6 or 8 inches deep prepared alongside the taproots of normal cotton plants grown in iron drums and tanks. The drums contained about 6 cubic feet of soil free from root rot, and two cotton plants were grown in each. The tanks contained 30 to 50 cubic feet of soil, and each had four plants. Six out of nine inoculations made in this manner were effective in killing the plants inoculated in a period of 5 to 16 days. (Fig. 1.) The disease was immediately communicated to the adjacent plants in the same containers, and several of them died within a few days. The fungus was reisolated from the first plant that died and was cultured in tubes and fruit jars. Seven cotton plants in a field free from root rot were inoculated in the same manner about September 1. Five of these died before October 2, and the disease was transmitted to adjacent plants. Large numbers of field inoculations were also made at various times during the summer, using one or two 4-inch sections from the taproots of recently infected plants. These were effective in killing 12 to 27 per cent of the treated plants.

In the light of these results there seems to be no doubt that under natural conditions in the soil the fungus is capable of attacking the living tissues of healthy plants after subsisting for long periods on dead tissues only.

GRASS AND DATE PALM HOSTS OF THE FUNGUS

Though some students of the root-rot disease have suspected that the fungus might live on the roots of grasses or other monocotyledonous plants and thus remain in the soil for long periods without killing any plants, there seems to have been no evidence to support this theory until June, 1928, when the writers discovered the root-rot mycelium extensively distributed over the roots of date palms at Sacaton, Ariz., and in California. (Fig. 2.) A short time later it was found on the roots of Johnson grass and Bermuda grass in proximity to dying alfalfa plants. On the larger date-palm roots and on the sheaths and rootstocks of Johnson grass the strands were firmly attached, creeping over the surface in the same characteristic manner as on the roots of the most susceptible hosts. On the fibrous roots of the grasses the strands for the most part ran over the surface longitudinally and were not easily detected.

In one date orchard in California the root-rot mycelium was found in abundance as deep as 4 feet on many of the roots of the date palms in the outside row. No live roots other than those of the date were found in the extensive excavations that were made in this orchard,

but a trench dug about 25 feet outside the infected row of date palms disclosed the fact that the infection originated from a row of almond and fig trees which once stood in what is now a roadway, adjacent to the date planting. These trees had been dug one at a time as they were killed by root rot through a period of several years, the last almond tree having been removed in 1927. Many decayed roots of these old trees were exhumed in digging the trench, and active mycelium was found pervading the decomposed material even at a depth of 4 feet. Frequently live date roots had penetrated through the old soft decayed roots, and invariably these were covered with root-rot strands.

Another observation on the relationship of grass crops to the root-rot disease was made on the Indian School farm at Sacaton in July, 1928. A 10-acre alfalfa field in which practically all of the plants had been killed by root rot was plowed in 1924 and planted to grain sorghums. Three successive crops of sorghums were grown, and the field was replanted to alfalfa in the winter of 1927-28. During the early summer months of 1928 frequent observations were made in this field, so that the first indications of recurrence of the root-rot disease



FIGURE 2.—Date root with a coarse strand of *Phymatotrichum omnivorum* growing along the surface and producing numerous fine strands

might be detected. Several small circular areas of dying plants were noted on July 20. Near the centers of several of these infected spots were clusters of young sorghum shoots which originated from old sorghum root crowns that had been turned over by the plow in breaking the land, but not killed. Some of these old crowns were split open and large root-rot strands were found creeping over the partly decayed tissues on the inside.

SELECTIVE FEEDING OF THE ROOT-ROT FUNGUS ON ROOTS OF DIFFERENT PLANT SPECIES

Several investigators (1, 3, 9, 13, 16) have reported that the root-rot fungus grows well on cooked vegetables and various other media, but apparently no serious attempts have been made to determine to what extent the organism shows discrimination in the types of plant materials on which it will grow or to ascertain whether there are any kinds of roots on which it fails to develop. After finding the mycelium in nature occurring in great abundance on the roots of

date palms and some of the common grasses, the writers attempted to grow the fungus on sterilized roots of these plants. Small pieces of pseudosclerotia were transferred from pure cultures to a series of tubes containing cooked roots of date, Johnson grass, Bermuda grass, barley, and corn. The fungus developed rapidly on the date roots and covered the contents of the tubes in a few days. On the roots of Johnson grass and Bermuda grass the development was much slower, but growth was continuous in some sealed tubes for three or four months, and all of the roots became enveloped with mycelium. The growth on barley and corn roots was extremely slow, but continued for several months.

When the cultures were 81 days old the contents of one tube each of date roots, Johnson-grass roots, and Bermuda-grass roots were transferred to sterilized quart fruit jars, each containing a layer of cotton roots on top of a 5-inch layer of moist sand. A vigorous growth resulted, and in a short time the mycelium had entirely covered the roots and had invaded almost every portion of the sand.

In order to obviate the chemical changes in the roots brought about by steam sterilization and to have the nutritive material in a state as nearly identical as possible with that found in nature, a series of tubes was prepared containing date roots that had been sterilized by immersion for a few seconds in alcohol. Inoculum material from pure cultures on date roots was added to these tubes, and the mycelium soon spread to the new material and grew vigorously for several weeks.

In comparing the development of mycelium on cultures of various roots it was observed that in tubes containing fresh citrus roots the mycelium failed to grow in contact with the citrus tissues, and if other nutrient material was present the hyphae avoided contact with the citrus roots and grew only on the other material. (Fig. 3.) Repeated trials were made and larger containers were used, but the results were always the same. No growth resulted on fresh sterilized tissues of citrus roots. This behavior seems somewhat significant in that citrus is one of the few orchard crops that is reported to have a high degree of resistance to root rot. It has been reported from Texas that young citrus trees have been killed by the disease, although well-established trees have not been known to die.

The fungus made the most rapid development on cooked roots of such plants as cotton, alfalfa, cowpea, peach, and *Malva parviflora*. The growth was much slower on roots of tamarisk, pomegranate, peppertree, mesquite, groundcherry, and sunflower. Upon these the pseudosclerotia were slower in developing.

DEPTH OF FUNGUS ACTIVITY

The depth at which the root-rot fungus may function would seem to be a matter of importance in connection with any control measures that are undertaken. However, it appears that no systematic studies have been made along this line. McNamara and Hooton³ report that open trenches 2 feet deep, prepared in advance of the disease, were effective in checking its progress in a cotton field. Prior to 1928 the writers had found the mycelium on cotton roots to a depth of 36

³ McNAMARA, H. C., and HOOTON, D. R. STUDIES OF COTTON ROOT ROT AT GREENVILLE, TEXAS. [Unpublished manuscript.]

inches and on peach roots to a depth of 40 inches, but most observations have shown that it seldom destroyed or was present on the roots of closely planted field crops at a depth greater than 30 inches. In July, 1928, an extensive series of trenches was made around an infected area in California in an attempt to isolate the disease and prevent its spread to new areas. On the east side of what was thought to be the infected area no indicator plants were present, but one of the trenches, which was 414 feet long and 3 feet deep, was contiguous to a date orchard in which clean cultivation was practiced. A few



FIGURE 3.—View looking down into a culture tube containing two types of plant roots as food media, with a pure culture of *Phymatotrichum omnivorum*. Two sections of citrus root are seen at the left, surrounded but not touched by the white mycelium of the root-rot fungus which developed from two sections of cotton root at the right. The cotton sections became completely hidden by the mycelium, while the citrus sections remained exposed for their entire length. $\times 4\frac{1}{2}$

feet on the other side of the trench was a bare roadway in which a row of almond trees and fig trees had once stood, but which had been dug one at a time as they were killed by root rot, the last being removed in 1927. Shortly after the trench was dug it was filled with water, and a few days later numerous conidial mats appeared on its walls, some at the bottom, 3 feet from the surface. The locations of the almond and fig trees were clearly defined by large patches of these mats which produced almost solid formations several square feet in extent wherever the trench transected numerous dead tree roots close together. The depth of the trench was increased in places to 6 feet,

and in the excavations root-rot strands were found on the dead roots of almond and fig and the live roots of date at a depth of more than 4 feet.

Soon after the trench was deepened it was irrigated again, and a new crop of spore mats appeared, the lowest at a depth of $4\frac{1}{2}$ feet. The mats spotted both walls of the trench through most of its length of 414 feet, with two short nonspotted intervals amounting to 44 feet. In one place where the ditch transected rotted roots of a dead pistache tree that had been dug five years previously, both walls of the trench were covered with extensive patches of mats.

In another short trench dug 6 feet deep near a group of dead pistache trees, live strands of the fungus were found on the decomposed roots at a depth of $5\frac{3}{4}$ feet, and spore mats almost completely covered the walls of this trench to a depth of 5 feet. (Fig. 4.) Some weeks later the trench was filled again with water, and shortly afterwards a new crop of mats appeared. This time they extended over the old mats, covering the walls of the trench almost completely, and appeared even at the bottom of the trench 6 feet below the surface of the ground. In this way the trenches proved to be of much use in definitely locating the disease in this area in the absence of indicator plants on the surface. In every instance where the plants near the trenches indicated the disease, or wherever there was a history of the disease among orchard crops, and even where the trees had given place to bare roadways, spore mats appeared in abundance. None, however, were seen in the trenches in areas where the disease had never been in evidence.

BARRIER METHODS OF CONTROL

During the spring and summer of 1928 further trials were made with barriers in attempting to check the advance of the disease in infected spots in alfalfa fields. Instead of using open trenches, which were found to be impracticable under irrigation methods, the trenches were refilled with soil that had been mixed with about 1 part fuel oil by weight of oil to 10 of soil. In order to conserve oil and labor a narrow trenching spade 6 inches wide by 18 inches long was used in removing the soil. (Fig. 5.) The barrier experiments were conducted in alfalfa fields because the limits of the disease were so definitely marked in this crop and the spots were more circular in outline. The disease could thus be observed over a longer period than is possible in cotton fields. The trenches were approximately 3 feet deep and were placed 3 feet in advance of the active zone of disease, which was indicated by the rings of recently wilted plants. Two trenches, of semicircular shape and about 100 feet long, were prepared on May 15, before the disease had killed any plants. The zone of active mycelium, however, was easily ascertained by examining the roots on the outside margins of circular bare spots where plants had been killed in the previous season. A part of the old diseased area behind the active margins, where nearly all the alfalfa plants had been killed in previous years, was plowed and planted to cowpeas in order to observe any reverse movement of the fungus or any renewed centers of infection. On June 13 the length of one of the barriers was increased to 180 feet.

Within a month after the barriers were prepared the disease, as shown by dead plants, had reached the oiled earth of the 180-foot

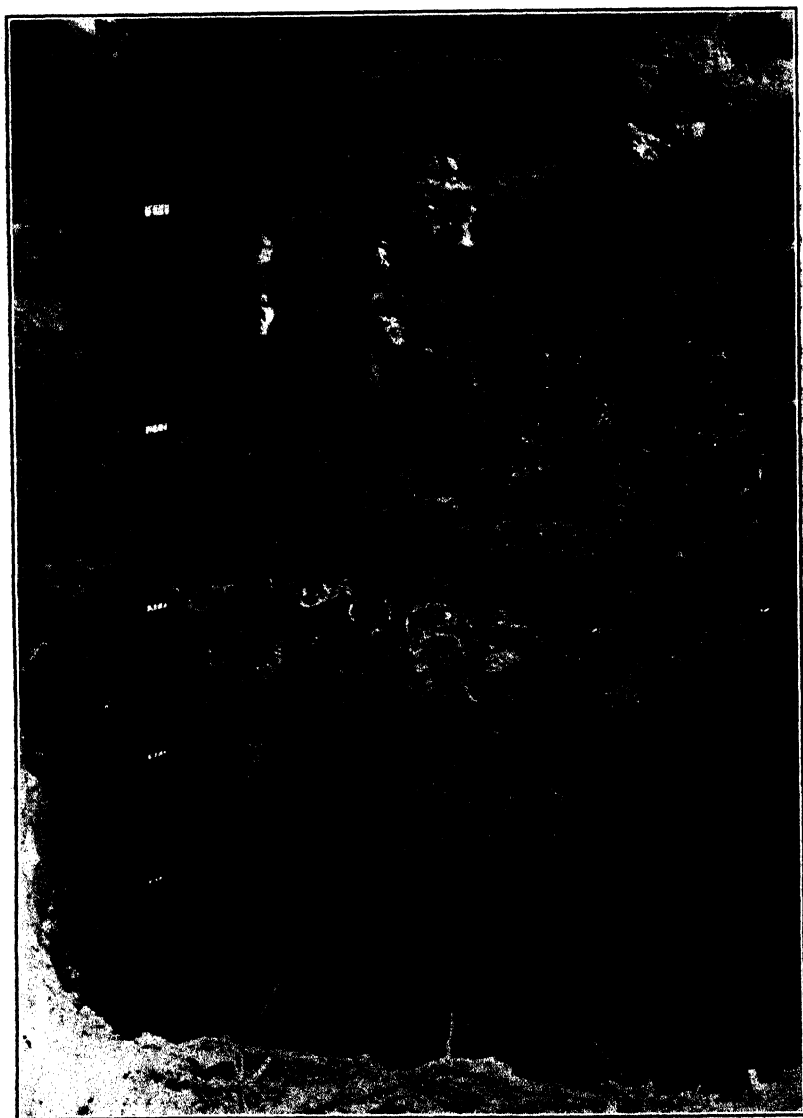


FIGURE 4.—Conidial mats of *Phymatotrichum omnivorum* lining the side of a trench 8 feet in depth. The mats which formed near the bottom of the trench had been obliterated by rodents at the time of photographing. The actively growing part of these masses is indicated by the white bands at the margins of the mats. In the same vicinity numerous formations of this kind appeared in trenches distant from any living plants, and where no live plant-root tissues were in evidence in the soil but dead root material was found in abundance.

trench in several places. On September 5 it had killed practically all of the plants behind this trench, but there was no indication of crossing over except in one place near the end where one or two



FIGURE 5.—Preparation of a barrier trench, to be filled with mixed oil and earth, about 3 feet in advance of the root-rot disease in an infected alfalfa field. The trench was dug with a narrow spade in order to conserve oil and labor

plants were dead on the outside of the barrier. (Fig. 6.) The disease was slower in reaching the oiled trench in the other spot, but on September 1 it was observed that only a few plants remained alive on the inside of the semicircular oiled barrier, and no dead plants were found on the outside.

On July 12 trenches were dug around two small circles of root rot in another alfalfa field. These were placed 3 feet outside of the band of recently dead plants and were dug only 26 inches deep. Long sheets of galvanized iron, 26 inches wide, were dropped on edge into these trenches, with the ends overlapping so that the diseased area

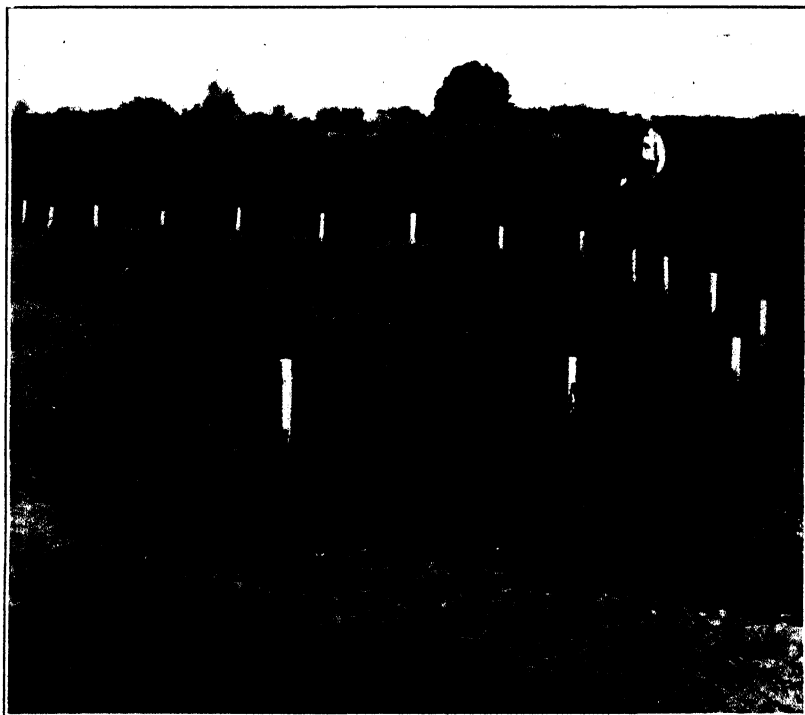


FIGURE 6.—A large root-rot spot in an alfalfa field where the spread of the disease was checked by an oiled-earth barrier 3 feet deep and 6 inches wide, indicated by the white stakes. The barrier was prepared on June 13, 3 feet in advance of the outer margin of the area infected the previous year. Nearly all of the alfalfa plants behind the barrier were killed before September 5, but none was infected on the outside of it when the spot was photographed on October 29

was completely encircled and isolated by an iron barrier 26 inches deep. The metal sheets were pressed firmly against the inside wall of the trench and the soil was replaced. All of the plants on the inside of the barriers were killed within five weeks, but in spite of the rather shallow depth of the barriers no plants on the outside were attacked, although the disease was active in other circles a few feet away for three months after its spread in the inclosed areas was checked by the barriers. Other barriers of oiled earth and galvanized iron were prepared on August 18, and at the end of the season no diseased plants were observed on the outside of the barriers, whereas all of those within had been killed long before the end of the season.

CLEAN-FALLOW EXPERIMENTS

It has been suggested by some investigators that the root-rot organism might be starved out by keeping the soil free from all living plant tissues for an extended period of time. McNamara (11) reported that a clean-fallow treatment for a period of two years at Greenville, Tex., was effective in controlling the disease. Later experiments at Greenville and similar experiments at other places in Texas, however, have not been so encouraging. Clean-fallow experiments were begun at Sacaton, Ariz., in 1925. An area of half an acre, comprising plots C3-1 and C3-2, on which the cotton crop of 1925 was badly infected, was maintained as a clean fallow during the years 1926 and 1927 and replanted to cotton in 1928. Plot C3-3, an adjacent area of one-fourth acre, on which only 3.5 per cent of the cotton plants were infected in 1925, was planted to corn in the summer of 1926 and to Canada field peas in the fall of 1926. In March, 1927, the peas, which showed no evidence of infection, were plowed under, and the area was maintained as a clean fallow until March 20, 1928, when it was replanted to cotton.

In maintaining the clean fallow, plots C3-1 and C3-2 were plowed once at the end of each summer after the initial breaking of the land, making three plowings in all. These were made with a moldboard plow which penetrated to a depth of 6 or 7 inches. Plot C3-3 was plowed early in 1926 to turn under the dead plants of the 1925 cotton crop, and a crop of cornstalks was also plowed under late in the same year. In 1927 this plot was plowed twice, the first plowing being necessary in March to turn under the crop of peas. In addition to the plowing operations, all three of the plots were gone over with a disk cultivator 8 or 10 times each year to keep down the growth of weeds and grass which came up after rains. Between diskings the plots were frequently inspected by laborers who removed all seedling weed growth and exhumed many tubers of *Rumex hymenosepalus* with hand implements.

During the months of June, July, and August, 1928, all of the cotton plants of these three plots were inspected daily, and a record was kept of all the separate points of infection that appeared, as indicated by the dying of plants at some distance from any that had died previously. The first dead plants were observed on June 17. The observations were continued until August 10, at which time 106 centers of infection had appeared on plot C3-1, 78 on plot C3-2, and 38 on plot C3-3.

During the early part of the summer an attempt was made to control the disease by digging out the fungus. The plots were inspected every morning, and plants that had wilted were immediately dug up, together with the adjacent plants on either side, if any infected roots were observed. The soil was removed to a depth of about 18 inches and hauled away. The sides and bottom of the excavations were then thoroughly sprayed with a strong disinfectant, and disease-free soil was brought in to refill the holes. By the middle of August it was apparent that little progress was being made by this method, as the disease continued to spread from about two-thirds of the treated centers, indicating that the advance mycelium had extended beyond the excavations; and the practice was discontinued.

On November 21 a survey was made of the diseased areas in these plots and a map was prepared showing the location of infected and

noninfected areas. The extent of infection in these plots in 1925 and after the fallow treatment in 1928 is shown in Figure 7.

The infected area, as shown in Figure 7, maintained approximately the same configuration after fallowing as before on plots C3-1 and C3-2, although the number of scattered patches of healthy plants in the large infected areas was greater in 1928 than in 1925. The percentage of infected area on plot C3-1 was 61.6, compared to 67.4 in 1925, whereas on plot C3-2 the infected area had increased from 30.5 to 41.3 per cent. On plot C3-3, which was fallowed only one year, following corn and a winter cover crop, the disease had increased considerably in extent, affecting the plants on 19.2 per cent of the area in 1928 as compared to 3.5 per cent in 1925.

It is apparent that the fallow treatment had no effect in reducing the amount of infection. The margins of the diseased areas in 1928

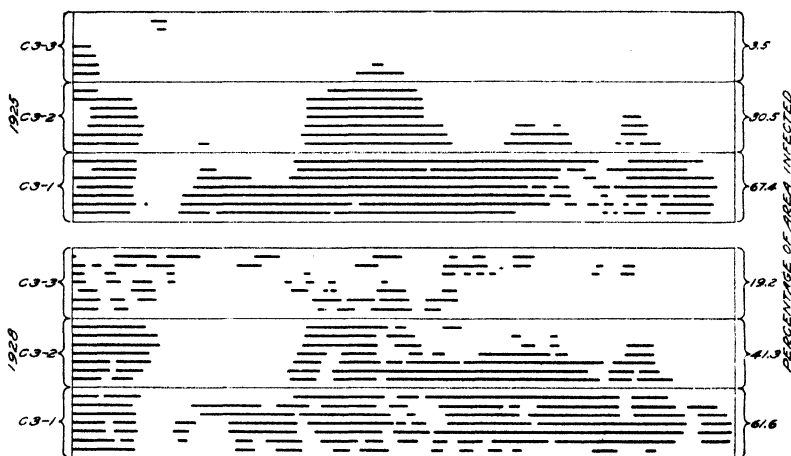


FIGURE 7.—Diagram showing the extent of root-rot infection on three quarter-acre plots of cotton in 1925 and 1928, before and after clean fallowing, at the United States Field Station, Sacaton, Ariz. Plots C3-1 and C3-2 were fallowed in 1926 and 1927, and plot C3-3 was fallowed in 1927, following successive crops of corn and winter peas in 1926. No material reduction of the area infected followed the fallow treatment, and the infected spots occupied about the same locations as before.

closely coincided with the margins in 1925, except that in most cases they had extended a few feet farther. This persistent behavior in the absence of any live host plants for a period of two years suggests that the organism was maintained under such conditions by some resistant form of mycelial development or that it can support itself from dead plant tissues. Extensive excavations were made after the 2-year fallow treatment to determine what types of plant tissues remained in the soil. Cross-section trenches were dug 2 to 3 feet deep at several places, but the only plant material exhumed was in small fragments and in such an advanced stage of decomposition as to be easily crumbled between the fingers. The cotton plants of the 1925 crop, as they were of small size, no doubt disintegrated more rapidly than ordinarily is the case under Arizona conditions. Since there was no evidence that any living tissues were present and as there was no dead material sufficiently large to give protection to a delicate mycelium, the evidence seems to indicate the presence of a highly resistant and resting form of the organism.

DEVELOPMENT OF PSEUDOSCLEROTIA

In 1893 Atkinson (2) reported sclerotiumlike bodies which developed in his cultures of *Ozonium* (*Phymatotrichum*). He described these as "from very small to 3 millimeters in diameter, whitish and woolly, finally becoming of the same color as the filaments of the fungus." Taubenhaus and Killough (16) also reported this form, which they termed "pseudosclerotia," as appearing singly or in large masses in cultures on various media and on host plants in natural surroundings. The writers have commonly observed the type which is found on infected roots (chiefly at the lenticels) and which several investigators (2, 4, 5, 12, 16) have described as "wartlike." However, the structure and size of the pseudosclerotial formations which develop commonly in artificial cultures are so different from these small accumulations of hyphae found in nature that it is difficult to consider them as analogous forms.

In pure cultures the growth of the pseudosclerotia depends on a properly moist food supply. With foods such as sweet potato, turnip, and the roots of cotton, alfalfa, and peach, on which the mycelium grows readily, pseudosclerotia may begin to form soon after the mycelium becomes well established on the roots. On foods on which the mycelium does not grow readily, pseudosclerotia may not form, or if they do it is very slowly, and only small masses are developed. They have been observed to form after several months on sterile roots of date palm, Bermuda grass, Johnson grass, and barley contained in culture tubes sealed with beeswax and paraffin.

In the ordinary culture tubes containing small sections of cotton or other suitable roots it has been observed that the pseudosclerotia may continue to develop until nearly all the space in the tube is occupied and the roots which supplied the food become shriveled and greatly reduced in size. In large containers, such as 1-quart or 2-quart fruit jars, containing sterile cotton roots and sand, single masses 20 millimeters or more in diameter and 5 or 6 millimeters in thickness have been developed, and the surface of the sand and roots has become almost completely covered by these masses. (Fig. 8.) These structures are obviously also aerial in character, as they form on the strands that have grown along the glass surface of the containers, at some distance from any food supply. They are also sometimes observed in cavities within the soil or sand cultures, but are apparently unable to develop where the soil is compact.

The pseudosclerotial structures become deflated in drying, and if allowed to remain in this condition for many months at ordinary room temperatures the hyphae become brittle, and in this state attempts to revive the fungus have thus far been unsuccessful.

A convenient method for multiplying cultures has been developed by the writers, making use of entire small fresh pseudosclerotia or fragments of the large ones. In the latter case enough material often can be obtained from a single body to propagate 25 or more cultures, and a quart fruit jar frequently affords sufficient material for several hundred cultures. These fragments seldom fail to grow, whereas failures are not uncommon where loose wefts of mycelium are used. In the incubator at 25° C. hyphae may begin to appear from the fragments in new cultures within three or four days.

Experiments to determine the length of time that these pseudosclerotia remain viable have not been completed, but it is apparent

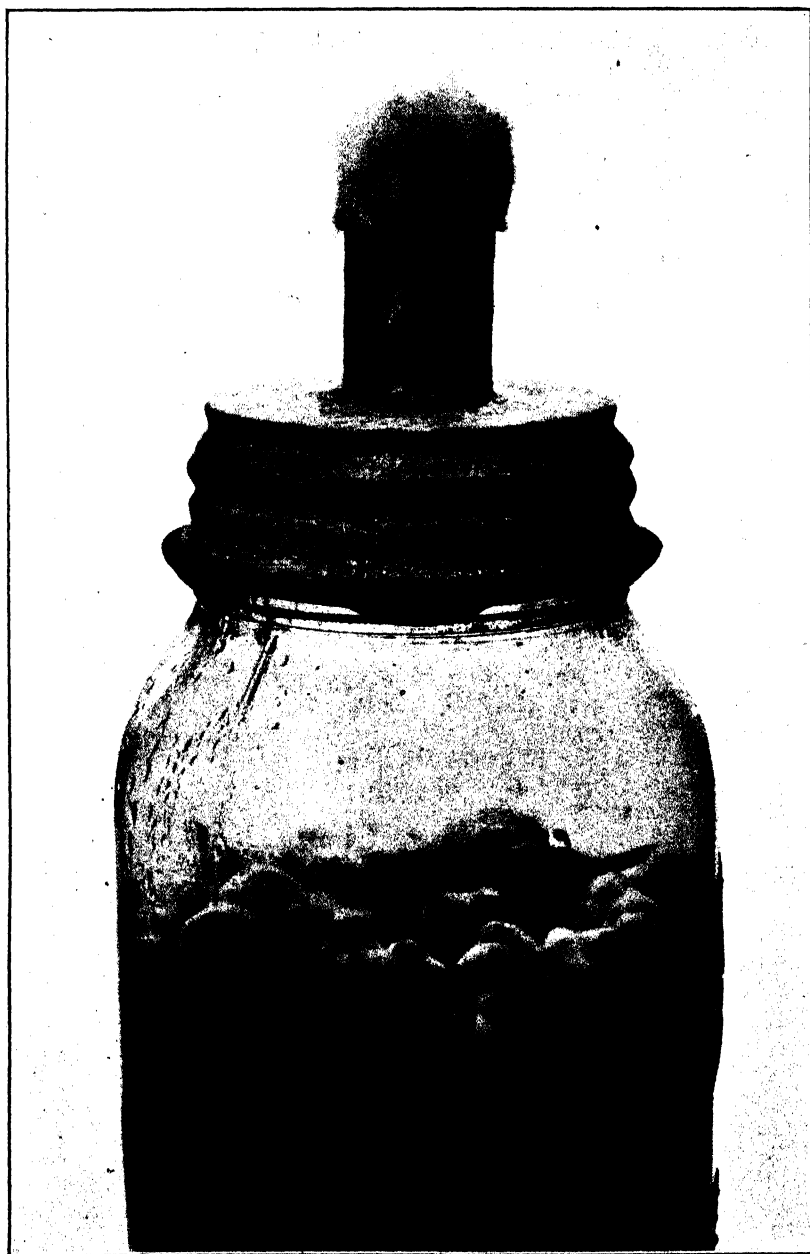


FIGURE 8.—Pure culture of *Phymatotrichum omnivorum* on dead cotton roots underlaid with moist sand, in a quart fruit jar equipped with a special lid. This jar was inoculated on October 4 with the fungus on a small section of date root from an 81-day-old date-root culture. The photograph shows the numerous large masses of pseudosclerotia which had developed by November 10 and almost completely hid the cotton roots and the surface of the sand. Such a culture contains sufficient pseudosclerotial material for preparing innumerable cultures of the fungus, and entire cultures of this type were used in inoculating healthy cotton plants

that under optimum conditions they may be kept for several months and still develop further mycelial growth when transferred to a fresh food supply.

A TRUE SCLEROTIAL STAGE OF PHYMATOTRICHUM OMNIVORUM

In describing the characters of the strand hyphae of the root-rot fungus, Duggar (3, p. 19) states that "they are more or less sclerotial and are doubtless an important factor in the persistence of the fungus in the soil." Taubenhaus and Killough (16, p. 73) apparently did not interpret this statement as applying to the strand hyphae primarily, as they comment on it as follows:

It is true that *Phymatotrichum omnivorum* does produce on the host and in pure culture sclerotialike bodies. These, however, are much of the nature of pseudosclerotia, and do not seem to be able to survive during the winter months on a dead host or in the soil. All our evidence on hand tends to show that *Phymatotrichum omnivorum* is unable to live as such in the soil as ordinary mycelium without the presence of a living host.

The writers have recently observed a hitherto undescribed type of mycelial structure which developed from the strands of the root-rot fungus in pure cultures with a substratum of sand or soil. This new structure is a form of sclerotium with characters that would enable it to live through the winter or through long periods in the field without a supply of food. The first development of these sclerotia was noted late in September, concurrently with unusually low temperatures. The fungus was being grown in long glass tubes containing only moist sterile sand, with the culture introduced at one end of the tube on a small section of dead cotton root. After the strands had grown several inches from the food supply the sclerotia began to form on the coarser portions of the strands. In October large numbers of these sclerotia were developed in pure cultures of *Phymatotrichum* raised in quart fruit jars on sections of cotton roots lying on 5 inches of sterile moist sand. Of 27 such cultures in fruit jars, sclerotia appeared in 21.

Prior to the formation of the sclerotia and during their development all of these jars were exposed to the ordinary temperatures of the laboratory, which at night occasionally dropped as low as 65° F., with day temperatures sometimes exceeding 95°. These bodies were produced either singly or in continuous series or in distinct clusters on the large strands that had penetrated the sand below the cotton roots. (Fig. 9, A, B.) Many sclerotia appeared on the strands that descended along the sides of the jars, and their development was plainly visible through the glass. Few were produced on the surface of the sand or on the mycelium above the sand. The formation of sclerotia was continued in some jars through the winter at room temperature of the unheated laboratory, a second crop of sclerotia being produced in a few jars. In these jars new sclerotia were developed in some of the old clusters and on some of the oldest strands, as well as on younger strands.

Sclerotia also developed in several jar cultures that had been kept for two or three months in an incubator held at a constant temperature of 25° C. and in other jars kept in a steam boiler room where the temperature fluctuated between 40° and 90° F. The fact that no sclerotia developed until September or October in the jar cultures prepared in the early summer, and their occurrence almost simul-

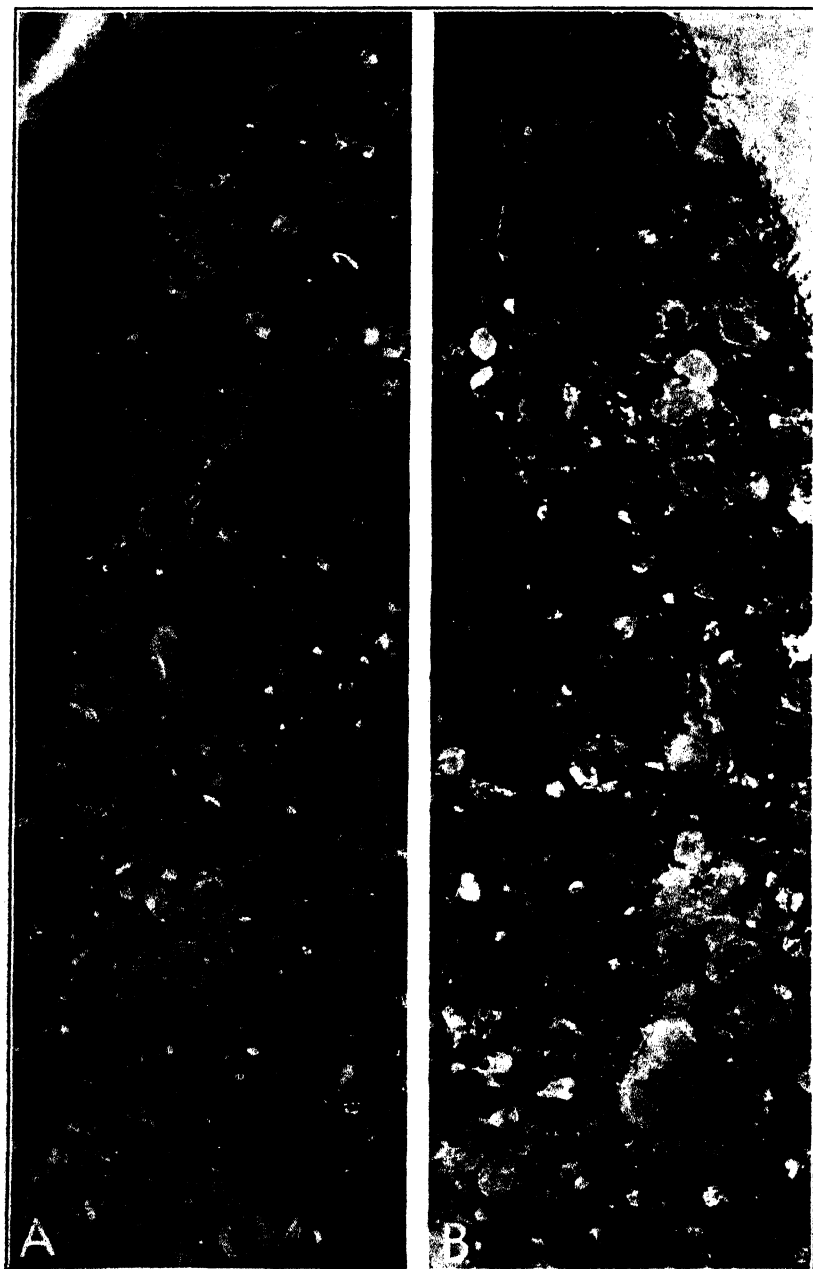


FIGURE 9.—Sclerotia developed on the strands of *Phymatotrichum omnivorum* in pure cultures in quart fruit jars. A, several large single sclerotia are shown in alignment in the middle of the photograph, while above and below them are compound formations resulting from sclerotia which started developing so close together on the strand as to form continuous series when fully grown. $\times 5$. B, conglomerate sclerotia produced on a single coarse strand of the fungus. These represent the largest masses that have been observed. Two young sclerotia may also be seen on the white strand near the upper left-hand corner of the photograph. $\times 5$

taneously in nearly all of the cultures, regardless of their age, when cool weather arrived, indicates that temperatures bear an important relationship to the development of sclerotia.

The sclerotia begin to develop as simple, white, elongate swellings of the larger mycelial strands (figs. 10 and 11; A), and maximum growth is attained within three or four days, with the fully developed sclerotia resembling miniature potatoes, round or ovoid in shape, and slowly changing in color through light yellow to reddish brown with age. The largest individuals were about 2 mm. in diameter, but many smaller ones were produced. In many cases the formation was so continuous along the strands that solid clusters were produced 10 mm. or more in length. The shape of some of the clusters also indicated that many of the sclerotia were developed by a budding process directly from the walls of other sclerotia (figs. 11, C, and 12), as well as from the strands themselves. (Fig. 13, A and B.) All of the sclerotia produced in two jars were carefully separated from the sand in order to count them. Although an entirely accurate count was not possible, because of the compound character of some of the clusters, it was found that at least 621 sclerotia had formed in one jar and 2,731 in the other. (Fig. 14.) The dry weight of the sand in each of these jars did not exceed 1,000 gm.

Microscopic examination of sections of the sclerotia showed that they were formed by the division and growth of the cells of portions of the large strands. This division of the cells apparently took place in the large central cells of the strands as well as in the smaller cells which surround the larger, as no continuous series of large cells of similar appearance extended through the sclerotia. (Fig. 11, A.) Sections show that the interior of the sclerotia is made up of closely packed, colorless, thin-walled cells of large and small size intermixed and varying in shape from roughly rounded to elongate oval and having no definite arrangement. (Fig. 11, B.) A view of the surface of the sclerotia presents a labyrinthine appearance caused by the very irregular or contorted shapes of the brown, moderately thick-walled cells. (Fig. 15, A, B.) From some of these surface cells, acicular hyphae, or setae, similar to those of the strands themselves, protrude at right angles, giving the sclerotia a somewhat bristly aspect under the microscope.

At the time of development of the sclerotia in the jars some of the cultures were nearly 3 months old while others were less than 4 weeks old. The inoculum used to establish the fungus in the jars in some cases consisted of pieces of pseudosclerotia while in others small sections of sterilized cotton roots, overgrown with the fungus, from type cultures, were introduced into the jars.

In a preliminary study of the viability of the sclerotia, several clusters were broken apart and the individual sclerotia placed on moist sterile cotton roots in culture tubes. The tubes were kept at a temperature of 25° C., and in a few hours a dense growth of fine, erect, white hyphae developed over the entire surface of the sclerotia in 32 of the 33 tubes. (Fig. 13, C.) More recent inoculation studies with sclerotia show that the above proportion of viability may be expected with great regularity when sclerotia are removed from cultures and placed immediately on a suitable medium.

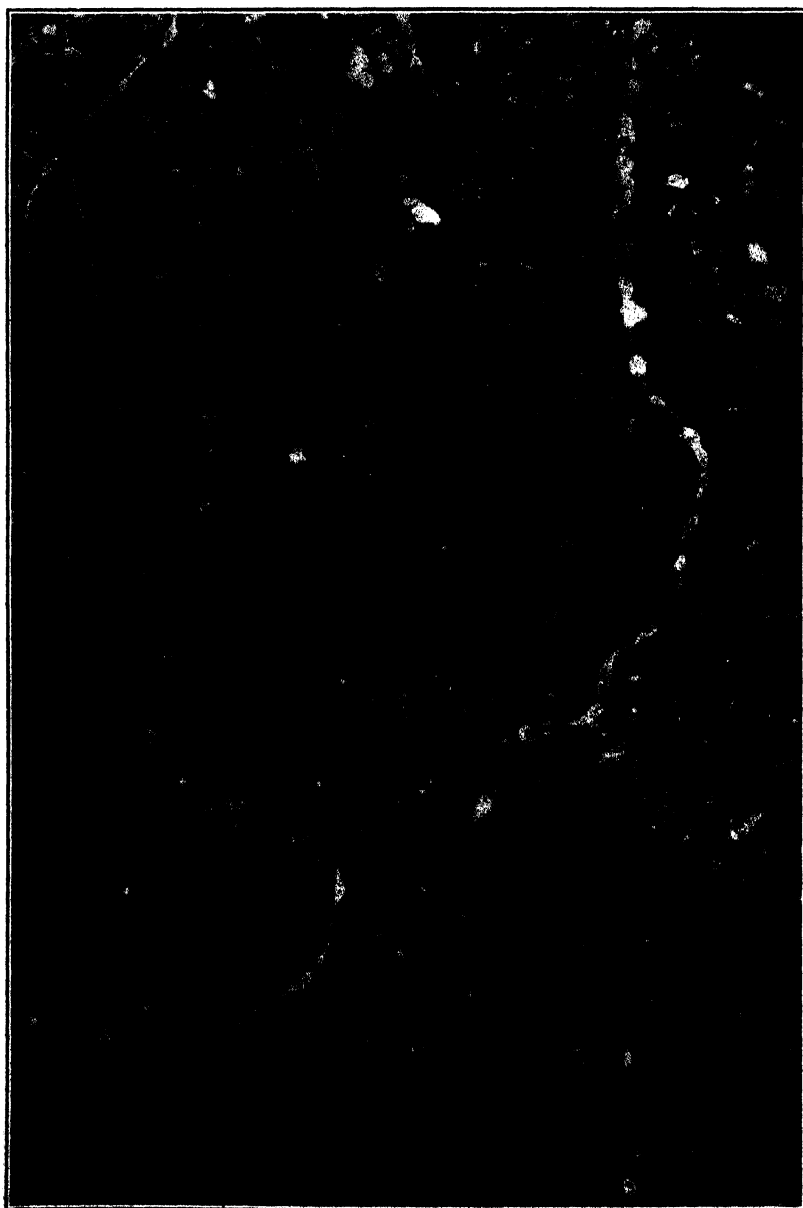


FIGURE 10.—Initial stages of sclerotial development on the coarse strands of *Phymatotrichum omnivorum* in pure culture in a large candy jar containing sand and dead cotton roots, showing the manner in which the sclerotia first appear as swellings of the large mycelial strands of the root-rot fungus. $\times 4\frac{1}{2}$



FIGURE 11.—Longitudinal sections of sclerotia of the root-rot fungus. A, a long portion of a strand which had begun developing into sclerotia. No semblance of the original strand remained except just above the middle of the long central portion of the sclerotial structure. $\times 30$. B, a single sclerotium, showing its connection with the strand on one side, and the shapes, sizes, and arrangement of the interior cells. $\times 58$. C, a large compound cluster of sclerotia illustrating the method by which other sclerotia bud from the sides of those produced directly from the strands. The junction of the cluster with the strand on each side is indicated by the small black arrows. $\times 32$

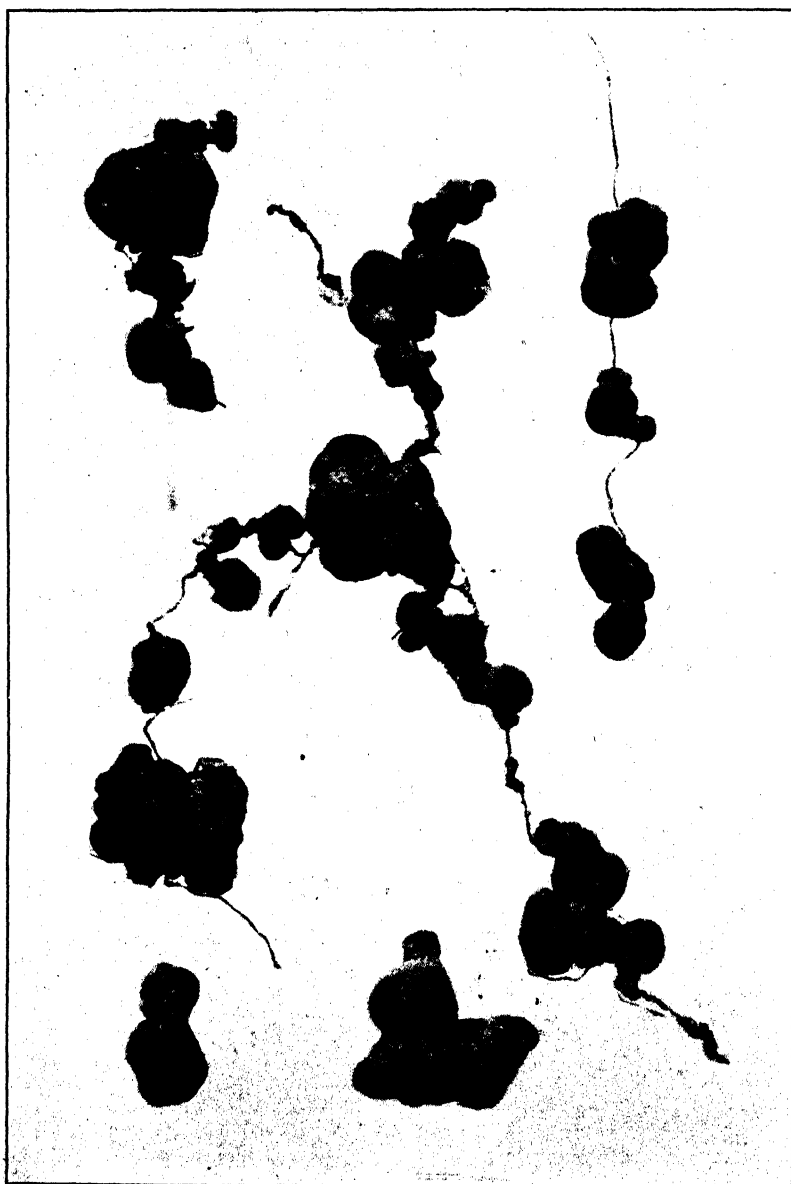


FIGURE 12.—Conglomerate masses of mature sclerotia of *Phymatotrichum omnivorum* removed from the sand in jars containing pure cultures of the fungus on dead cotton roots. The aggregate character of the sclerotia and their disposition on the strands will be noted. $\times 7\frac{1}{2}$

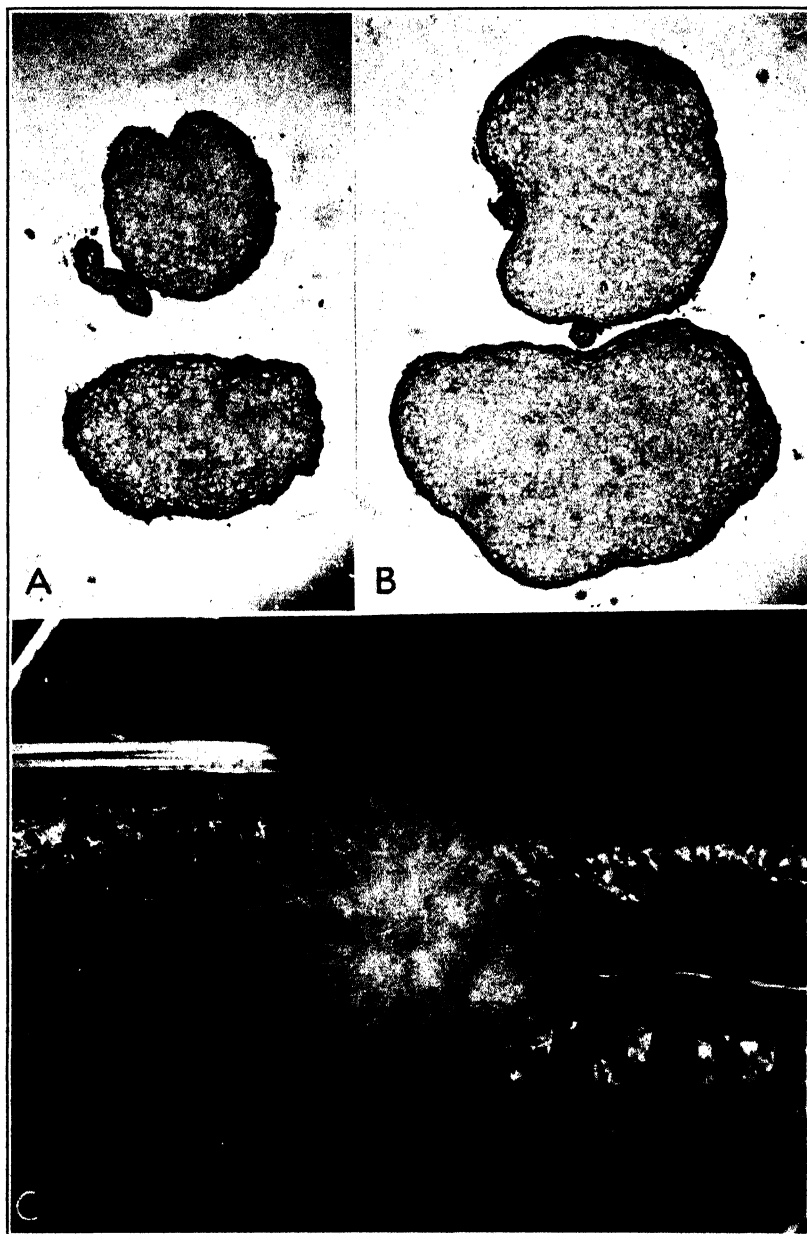


FIGURE 13.—A, Cross sections of two sclerotia and a short portion of the connecting strand. $\times 45$. B, other cross sections of the same two sclerotia showing the junction of one end of the intervening strand with the upper sclerotium, while the other end is indicated near the point of connection with the lower sclerotium. $\times 45$. C, single sclerotium germinating on dead cotton-root tissue in a culture tube incubated at 25° C. The dense growth of white hyphae developed in 21 hours. $\times 5$

Within 48 hours after one of the sclerotia is placed on a cotton-root plug in a culture tube the typical coarse strands begin to form and make possible the positive identification of the mycelium without the use of the microscope. After the coarse strands appear, the cotton roots are quickly enveloped with this type of growth. In

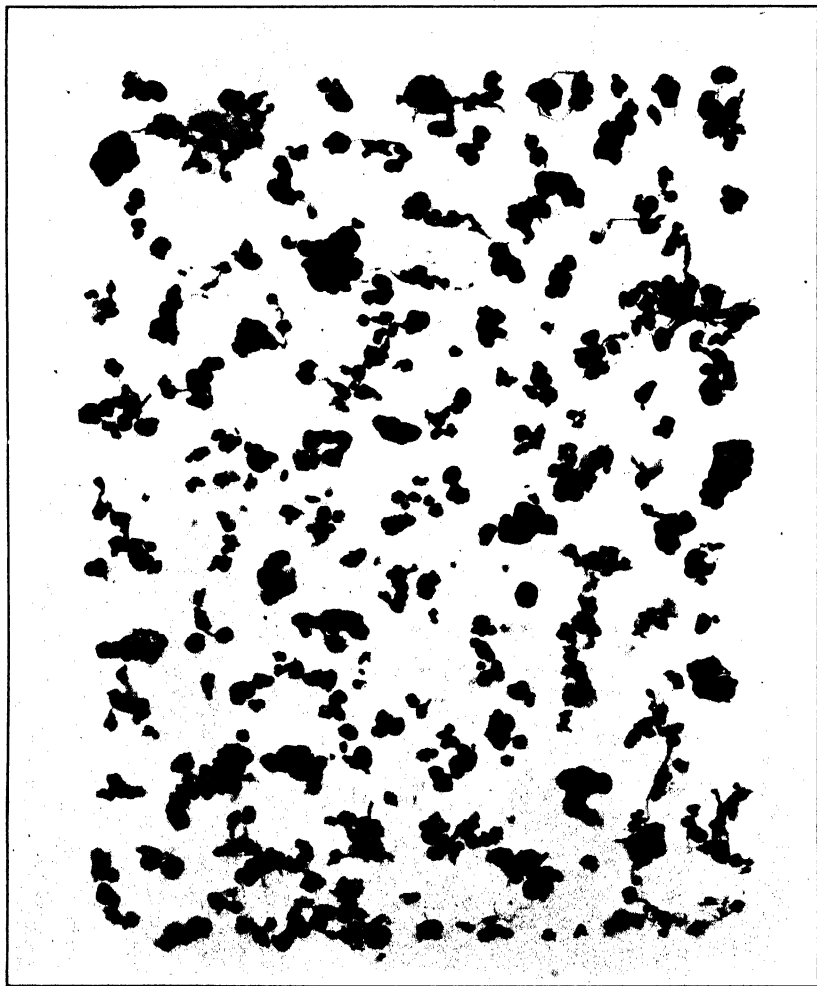


FIGURE 14.—Specimens of sclerotia of *Phymatotrichum omnivorum* representing about nine-tenths of the total number produced in the sand of a single quart fruit-jar culture. After separating the clusters into their component parts it was estimated that over 600 sclerotia, each capable of reproducing the active fungus, were produced in this small volume of sand, not exceeding 700 cubic centimeters. $\times 1\frac{1}{2}$

several fruit-jar cultures, containing pieces of cotton roots placed on moist sand and inoculated with a single sclerotium, the same rapid development of the fungus was observed. In less than two weeks pseudosclerotia began to form, and the surface of the sand and roots was covered with a dense and almost continuous formation of feltlike material.

On November 4 the root-rot fungus was established in two long glass tubes containing sand which had been washed with sulphuric acid to remove the organic food material. A single small true sclerotium was introduced at one end of each tube. Hyphae developed in a short time and began advancing along the tubes. The daily progress of the hyphae is given in Table 1, reference to which shows that the growth in one of the tubes was quite irregular throughout its entire course. The growth in the other tube was more uniform and extended over a longer period, with a general slowing down observable after November 10. However, the mycelium in this tube was able to advance a distance of nearly 4 inches on the quantity of food supplied by the single small sclerotium alone. Under field conditions, a similar movement of the mycelium from a disconnected sclerotium might be the means of bringing the fungus to a fresh supply of food.

Although tests indicate that the sclerotia are very short-lived when removed from cultures and dried in the air before being placed on media, there is proof that they are capable of withstanding other rigorous treatments. A large number of sclerotia were surface sterilized by immersion in an aqueous solution of 1:1,000 parts of mercuric chloride for 30

to 60 seconds, after which they were rinsed in sterile water and placed on moist sterile cotton roots. Without exception, the mycelium began developing from the sclerotia within 24 hours. Sclerotia more than 45 days old and a few others 70 days old, all of which had been exposed in the culture jars to temperatures as low as 50° F., showed no loss of vitality after being removed from the jars and placed on a medium of moist cotton roots. Other sclerotia from these cultures were completely immersed in sterile water at room temperature for periods varying in length from 1 to 28 days. After being immersed for 28 days, four sclerotia were placed on cotton roots in culture tubes in the incubator and 18 hours later three of the four began developing hyphae which were easily visible to the eye. This ability of the sclerotia to live for long periods under

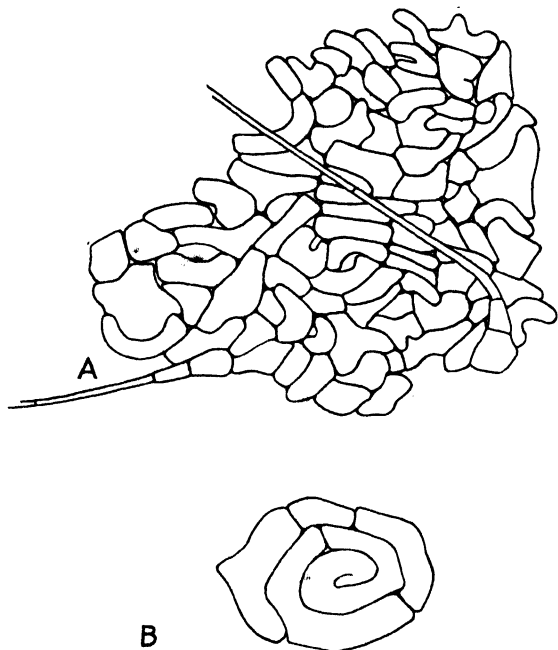


FIGURE 15.—Surface cells of a sclerotium of the root-rot fungus. A, showing the great irregularity of the size and shape of the surface cells and the development of the slender, acicular hyphae. $\times 350$; B, series of cells with a somewhat spiral arrangement, of rather frequent occurrence on the surface of the sclerotia and largely responsible for its labyrinthine appearance. $\times 575$

water may explain the survival of the root rot in areas that have been flooded for weeks or months in an effort to drown out the disease. Several instances of such persistence of the disease after flooding have been observed by the writers. In one instance water several inches deep was held continuously on a field in the Salt River Valley for 62 days during the winter, yet several spots of infection were noted in the cotton grown in this field the following summer.

TABLE 1.—Daily growth of strands of *Phymatotrichum omnivorum* in glass tubes (3 feet long and 1 inch in diameter) filled with acid-washed sand and inoculated with a single true sclerotium on November 4, 1928

Date	Growth		Date	Growth	
	Tube 1	Tube 2		Tube 1	Tube 2
	Mm.	Mm.		Mm.	Mm.
November 6.....		13	November 14.....	10	8
7.....	25		15.....	8	6
8.....		19	16.....	2	4
9.....	5	14	17.....		5
10.....		12	18.....		2
11.....		5			
12.....		6	Total growth.....	58	98
13.....	8	4			

Since the discovery of the sclerotia, many specimens have been used to establish cultures of the root-rot fungus, and their superiority over other parts of the fungus for this purpose has been demonstrated in several particulars. The fungus may be established in practically 100 per cent of the cultures inoculated with sclerotia, a much greater proportion than the writers have been able to obtain by any other means. The incipient growth of the mycelium on the nutrient medium takes place much more rapidly from sclerotia than from other parts of the fungus. A better opportunity is afforded for excluding contaminations from the cultures, as the surface of the sclerotia can be sterilized with antiseptic solutions before they are placed on the nutrient medium.

Efforts were made in several fields to find these sclerotia in the soil, but such efforts have been fruitless. There can be little doubt, however, that sclerotia occur in nature, although special requirements of food, moisture, and temperature may be necessary for their formation. From the evidence at hand it seems likely that they develop about the time the disease ceases to be destructive, with the approach of cold weather in the fall. The disappointing results that have been obtained in many places with chemical and cultural methods of control suggest the presence of a resistant form of the fungus, such as these sclerotia, which doubtless are capable of withstanding more severe treatments than the mycelium. It seems not improbable that the sclerotia may be responsible for the overwintering of the disease, at least in some parts of the infected regions.

MEASURING THE GROWTH OF MYCELIAL STRANDS

Taubenhaus and Killough (16) state that "Texas root rot spreads underground from contact of infected roots," and that otherwise "we should find hundreds of plants dying all at once in a given spot." Peltier, King, and Samson (13) are not in agreement with this view

and point out that the spread of the disease is through the agency of the advance mycelial strands, which are "present in advance of the wilting plants, while the mycelium in the zone of wilted plants serves as a food reserve for the advancing strand hyphae." Although the writers have been aware from field observations and from studying exhumed root systems of affected plants at various stages of infection, that the preponderance of evidence tended to support the latter theory of advance by mycelial strands, it seemed desirable to study this phase of the problem in greater detail on account of its important bearing on the development of control measures.

It was known from laboratory experiments that the mycelium grew readily on sterilized cotton-plant tissues, regardless of whether they were fresh or old and badly decomposed, and that its strands showed a tendency to grow in all directions from its food supply where moisture was afforded. An illustration of this tendency was furnished by a pure culture on a small piece of dead cotton root in a covered Petri dish kept in a 10-inch moist chamber on a large sheet of moist blotting paper on top of a laboratory worktable. After preparation, the culture was given no attention for several days. Then it was observed that the mycelium had escaped from the Petri dish, had grown several inches across the filter paper covering the bottom of the moist chamber, had climbed over the 2½-inch side of the lower section of the chamber, and had spread in its characteristic manner for 4 or 5 inches over the large sheet of moist blotting paper. After witnessing this and similar behavior, and after demonstrating that the strands grew readily from one end to the other of columns of moist sand or soil in student-lamp chimneys, the writers resorted to glass tubes 1 inch in diameter and from 30 to 36 inches in length for studying the elongation of the mycelial strands.

Satisfactory sterilization of these glass tubes was accomplished by filling them with alcohol and allowing this to remain for a few minutes before removal. They were then dried, flamed, and filled with the sterile soil or sand. After trial of various types of soil, it was found that the mycelial strands grew most rapidly in pure sand and were observed therein more easily than in soils of finer texture. Greater ease of preparing the glass-tube cultures also attended the use of sand, as the tubes could be filled to a more uniform density than was possible with moist soil, and the optimum moisture content could be more easily maintained. The sand was thoroughly sterilized in an autoclave before being placed in the tubes, and after the tubes were filled the moisture content was adjusted by standing the tubes upright and pouring sterile water in the upper end, allowing the excess to drain out below. After this the tubes were kept in a horizontal position, and the movement of water in the sand was negligible and offered no complications. When the correct moisture content was reached a section of cotton root covered with active strands from a pure culture was placed in one end of the tube which was then stoppered with a sterile cork. Several days usually were required before the mycelium began to grow through the sand and along the sides of the tube, where it could be observed easily by the use of a hand lens. The daily progress of the fungus along the tube was marked on the glass with a china-marking pencil, and the growth was thus measured and recorded.

The daily growth of the mycelial strands in the long glass tubes filled with sand, the treatments that the sand had received, and the

dates of the observations are shown in Table 2. Tubes 1 and 2 were filled with water-washed sand and were inoculated on August 23 with pieces of dead cotton roots, about $\frac{1}{4}$ by $1\frac{1}{2}$ inches long, covered with a pure culture of *Phymatotrichum omnivorum*. The first elongation of the strands in tube 1 was observed on August 28, and in tube 2 on August 27. The ensuing growth in tube 1 appears to have been somewhat erratic for the first week, but this was due mostly to the fact that part of the mycelial growth occurred in the sand and was not visible for accurate measurements. Tube 2 had a more uniform growth of the fungus until after September 8, and on this date the piece of cotton root was removed from the tube in an effort to ascertain whether the growth of the fungus would be affected. Two days after the removal of the cotton root the elongation of the strands appeared to slacken, but later it continued at nearly the same rate as before. Though a slowing up of the growth actually took place, a part of the irregularities in the measurements may be ascribed to the fact that at times the most advanced portion of the strands was obscured in the sand and could not be accurately traced. Later, the daily advance of the strands was more easily discernible, and the growth continued at a rate nearly uniform and but little less than that in the control (tube 1) where the food supply

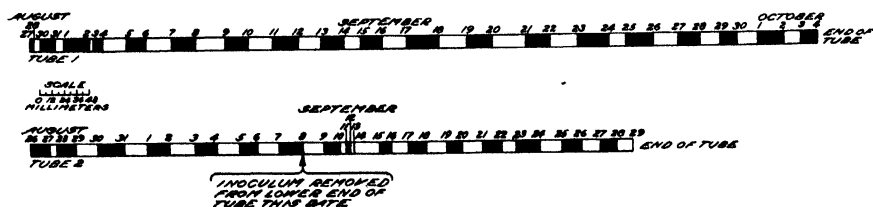


FIGURE 16.—Daily growth of the root-rot strands in long glass tubes, 1 inch in diameter, filled with water-washed sand and inoculated with the fungus on pieces of infected dead cotton roots at the lower end of the tube. In tube 1 the strands grew a distance of 764 millimeters and were stopped by reaching the end of the tube. In tube 2 the strands reached the end of the tube after growing a distance of 584 millimeters, of which 319 millimeters took place after September 8, after the piece of infected cotton root which was used to establish the fungus was removed from the tube

was not removed. The growth of the fungus continued through the whole length of the tubes, 30 inches in tube 1 and 23 inches in tube 2. In tube 2 the mycelium grew more than 12 inches after the removal of the piece of cotton root which served as a food supply to the fungus while establishing itself in the tube. The daily growth of the mycelium in these tubes is graphically shown in Figure 16.

In considering the behavior of the fungus in tube 2 after the removal of its original food supply, the idea was suggested that the sand in which the fungus was growing might contain organic matter which continued to furnish nutriment to the fungus after the removal of the cotton root. In order to determine whether such was the case, a quantity of sand was screened and immersed in a strong solution of sulphuric acid for two and one-half hours to remove the organic matter. It was then washed, sterilized by steam heat, placed in the tubes, and inoculated with the fungus on cotton roots in the same manner as was described for tubes 1 and 2. After the strands had advanced several inches through the sand the cotton roots were removed from some of the tubes, but in no case was growth stopped or more than slightly retarded.

TABLE 2.—Daily growth of strands of *Phymatotrichum omnivorum* in glass tubes 1 to 6 (each 3 feet long and 1 inch in diameter) filled with sand, and inoculated with the fungus on pieces of dead cotton roots in 1928

Date	Growth in sand washed in water		Date	Growth in sand washed in H ₂ SO ₄		Date	Growth in sand washed in H ₂ SO ₄ , tube 5	Date	Growth in sand washed in H ₂ SO ₄ , tube 6
	Tube 1	Tube 2		Tube 3	Tube 4				
	Mm.	Mm.		Mm.	Mm.		Mm.		Mm.
Aug. 27		20	Sept. 21	12		Oct. 6	6	Oct. 30	13
28	5	7	22	26	14	7	11	31	13
29		18	23	16	18	8	8	Nov. 1	21
30	6	21	24	34	29	9	10	2	14
31	14	25	25	24	24	10	11	3	26
Sept. 1	8	23	26	21	15	11	14	4	25
2	26	22	27	22	23	12	18	5	21
3	3	24	28	15	17	13	14	6	20
4	8	21	29	20	^a 16	14	12	7	28
5	23	22	30	28	25	15	9	8	23
6	20	18	Oct. 1	22	20	16	9	9	33
7	25	22	2	22	24	17	10	10	28
8	23	^a 22	3	18	17	18	5	11	25
9	28	21	4	18	17	19	4	12	33
10	23	15	5	16	14	20	5	13	27
11	24	7	6	12	18	21	3	14	26
12	25	4	7	18	16	22	8	15	25
13	22	3	8	^a 14	7	23	11	16	27
14	23	1	9	11	^a 3	24	2	17	20
15	16	23	10	12	18	25	12	18	5
16	19	12	11	15	16	26	8	19	11
17	24	16	12	11	11	27	10	20	20
18	32	17	13	8	10	28	10	21	15
19	28	20	14	4	5	29	18	22	22
20	25	16	15	3	3	30	23	23	35
21	32	19	16	2	3	31	16	24	20
22	22	18	17	^(b)	2	Nov. 1	14	25	32
23	27	15	18		1	2	14	26	21
24	31	22	19	4	3	3	13	27	25
25	16	21	20	^(b)	4	4	14	28	30
26	27	19	21		6	5	15	29	30
27	23	18	22		6	6	19	30	15
28	21	17	23		3	7	19	Dec. 1	19
29	20	15	24		3	8	16	2	20
30	17	^(c)	25		^(b)	9	17	3	20
Oct. 1	21					10	14	4	24
2	23					11	19	5	15
3	17					12	23	6	13
4	17					13	8	7	10
5	^(c)					14	17	8	10
						15	10		^(c)
						16	4		
						17	3		
						18	2		
						19			
						20	2		
						^(b)			
Total growth.	704	584		428	421		510		860

^a The piece of cotton root which supplied the food to the fungus in establishing itself in the tube was removed after the growth was recorded on this day.

^b Growth ceased.

^c End of tube.

The daily growth of the fungous filaments through the acid-washed sand in tubes 3 and 4 is shown in Table 2. In tube 4 the cotton-root plug was removed eight days after growth began, whereas in tube 3 the cotton-root plug was left, as a control. As will be seen in this table, or by reference to Figure 17, which illustrates graphically the progress of the strands in the tubes, the growth of the fungus was remarkably even in both tubes throughout the period of activity, with no retarding effect observable following the removal of the piece of cotton root from tube 4, as had occurred in tube 2. The terminal growth in the two tubes, as shown in Figure 17, was arbitrarily equalized on September 29, the day on which the cotton root was

removed from tube 4. The daily growth rate of the strands thereafter is seen to have been almost identical in the two tubes, the growth in tube 3 leading that in the other tube very slightly until October 13, when the growth in tube 4 caught up with that in tube 3 and thereafter exceeded it, both in the rate of elongation of the strands and the period of its activity. Late in October a period of low temperatures retarded and finally stopped the growth of the fungus in these tubes.

The most satisfactory explanation that seems to be offered for the continuation of growth of the strands in the tubes after the removal of the pieces of cotton roots is that it is a habit of the fungus to translocate nutrients rapidly from the older portions of the strands for the development of peripheral growth.

In another tube (No. 5 in Table 2) elongation of the strands was maintained for 46 days. This tube, which contained acid-treated sand, was inoculated on October 3. The first growth was detected on October 6, and continued until November 20. From October 6 to October 24 the tube was kept at ordinary room temperature, which, toward the end of this period, occasionally fell below 65° F. at night and caused a decided retardation of growth. On October 24 the

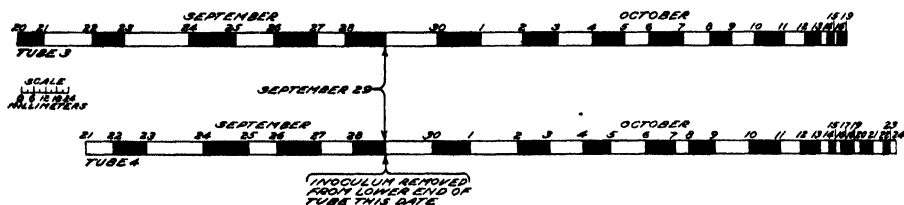


FIGURE 17.—Daily growth of root-rot strands in long glass tubes, 1 inch in diameter, filled with moist sand washed with strong sulphuric acid to remove the organic matter, and inoculated with the fungus on pieces of infected dead cotton roots at the lower end of the tubes. In tube 3, used as a control, the growth of the strands was both rapid and sustained until late in October. The infected cotton-root section was removed from tube 4 following the measurement of growth of the strands on September 29. It will be observed that no effect on the elongation of the strands is apparent after the removal of the original food supply.

tube was placed in a thermostat-controlled incubator with a constant temperature of 25° C. An increase in the growth rate immediately took place and was maintained for three weeks, after which the growth began to slacken, and it stopped on November 20.

Another tube of acid-washed sand, here designated as tube 6, was inoculated on October 26 and was immediately placed in the incubator. The first growth of mycelium was noted on October 30, and this continued until the other end of the tube was reached on December 8. The daily increase in growth is shown in Table 2. With the exception of a short interval beginning November 18, a very uniform growth was maintained to near the end of the tube, where it was retarded, apparently by a somewhat drier condition of the sand. On several days the advance of mycelium through the tube exceeded 1 inch. In only one other instance was a greater advance made in a single day, a growth of 42 millimeters having been recorded in another tube in a previous experiment. The mycelium in tube 6 advanced a total distance of 860 millimeters, or approximately 34 inches, from the piece of cotton root which supplied food to the fungus and which was not removed from the tube.

REACTIONS WITH OTHER FUNGI

An exception to the continuously uniform elongation of the mycelium in tube 6 occurred between November 17 and 21, when the growth on one of these days decreased to 5 millimeters. This slowing down of growth occurred at a point about midway in the tube where a contamination in the form of a fungus which resembled a species of *Fusarium* pervaded the same column for a little over 2 inches of the length of the tube. The root-rot mycelium reached this contamination some time prior to the measurement of growth on November 17, but the maximum inhibitory action was apparent the following day and continued somewhat in effect for several days. However, after the contamination was passed the root-rot mycelium resumed growth at practically the same rate as before the secondary organism was reached.

Many cases of the inhibitory action of other fungi or of bacteria in cultures of *Phymatotrichum omnivorum* have been observed. Frequently the presence of another fungus in the long glass tubes containing sand, or in culture tubes, entirely checked the development of the root-rot mycelium, and in agar cultures the presence of colonies of bacteria retarded or entirely stopped the development of *Phymatotrichum*. This inhibitory action of specific fungi or bacteria suggests that other soil organisms may be a factor in the distribution of the disease and may account somewhat for the slow reinvasion of areas through which the disease has recently passed. It is commonly observed that root-rot injuries are generally followed by the appearance of other fungi, not capable in themselves of gaining entrance to living plants, and the activities of other fungi may hinder the immediate return of the root-rot fungus or discourage retrogressive movement of the strands.

EFFECTS OF VARIOUS PLANT TISSUES AND FLUIDS ON THE GROWTH OF ROOT-ROT MYCELIUM

The experiments with long glass tubes also made possible a preliminary study of the selective feeding of the fungus, as shown by the behavior of the strands visible in the tubes. A few tubes were filled with soil containing sterilized macerated roots of cotton, pomegranate, and citrus, and other tubes with soil moistened with sterilized sap that had been expressed from the leaves of the same plants. No growth of the fungus resulted from inoculations of the cultures containing the citrus and pomegranate products. However, the fungus did not fail to grow in any of the tubes containing macerated cotton roots, and it grew in one of the two tubes containing fluids of cotton-leaf tissue. The mycelium developed in these cultures was normal, and the elongation of the strands continued for a long period. Though the number of tubes included in this test was too small to give reliable information, the lack of growth of the fungus in the tubes containing citrus material is of interest in view of the results already mentioned in using citrus roots as culture media.

The uniform rate of elongation of the mycelial strands, when the fungus is cultured in columns of sand and maintained under similar conditions, affords an opportunity to study, with very simple equipment, the influence of various factors which may affect the growth of the fungus. The effect of temperature, moisture, chemicals, various plant tissues and fluids, competitive organisms, and other factors can be determined readily by this means.

OBSERVATIONS ON ROOT-ROT DISSEMINATION

The claim has been made by some investigators that root rot is spread from one field to another through fragments of fungus threads which are carried with soil particles by wind, or by the tools used in tillage, and by birds and animals. Warnings have been issued in regard to transferring soil from one field to another in inoculating for certain legume crops, and in the matter of distributing manures as fertilizers. Taubenhaus and Killough (16) are doubtful about the disease being spread by such methods as the above, but state that "it is safe to assume that the conidia, at least in the summer, do help to spread about the causal organism." The writers have never been able to obtain growth from detached fragments of strands from the soil, nor from those entirely separated from the surface of infected roots. Although attempts have been made by several investigators to germinate the conidial spores, there is as yet no record of a viable mycelium having been developed from them. On the basis of these facts and from observations made on infections in new areas and revived infection in old areas, it does not appear that the conidia play any important part in the dissemination of the disease. Nor is it believed that detached fragments of ordinary strands are a source of danger. There is, however, evidence to indicate that the disease can be transmitted to new areas on the roots of living plants, which may be susceptible to the disease or may serve only as carriers. Since there is good evidence that fragments of dead plant tissues harbor the mycelium in a viable condition, such tissues may be capable of conveying and transmitting the disease to new areas.

Although there is as yet no direct proof that the true sclerotia, as now shown to be developed in cultures, are produced under natural conditions in the soil, it is entirely reasonable to assume that they are, and this opinion may be supported by observations of the occurrence and dissemination of the disease. In preparations for growing ornamental plants about a pretentious winter home located on a rocky eminence near Superior, Ariz., a fertile soil was transported from the floor of a small stream bed which lay in a deep canyon at the rear of the residence. The fertile soil was placed in protected coves and depressions near the buildings. Many of the annuals planted in this soil were killed by the root-rot disease, which has persisted in these locations for several years. Plantings made later along the stream bed from which the soil was taken showed extensive root-rot infection.

Before the discovery of the sclerotial stage, the writers made several attempts to transmit the disease to new areas by transferring soil from infected areas, but all resulted in failure. These transfers were made during the summer months, with the precaution of taking the soil from the peripheral zone where the fungus was active. But from observations made in the laboratory it appears that the sclerotia may not be developed until late in the season, with the approach of winter.⁴ If this also is true in nature, soil samples obtained in the summer from recently infected areas might contain only mycelium, whereas winter samples of soil might carry sclerotia.

⁴ Subsequent experiments have shown that sclerotia may develop in cultures at any period of the year and under a wide range of temperature.

It may be true also that the development of sclerotia on old strands that remain in the soil for some time after the disease has passed would be interfered with to some extent by the activities of secondary organisms, and that the most extensive development of sclerotia would take place in the fall on the advance strands that are active on the edges of infected spots. This is indicated by the regularity with which the disease reappears in the spring on the margins of areas where the activity was last apparent. (Fig. 7.)

McNamara and Hooton⁵ report having observed in 1926 a formation of spore mats in a large root-rot spot in a cotton field in close arrangement within the area that had been infected by the disease in 1923, while only a few mats were found in the area covered by the advance of the fungus during the subsequent five years. Apparently no one has been able to offer a satisfactory explanation for this phenomenal behavior, but with a knowledge of the sclerotia and their physiology, now available, explanations can be offered which are entirely reasonable, although still in the realm of conjecture.

In spite of failures that have been made in attempting to transmit the disease by transferring soil from infected areas, it is the opinion of the writers that practices which involve transfers of soil, such as the balling of trees for replanting, and releveling of land under irrigation, in infected places, involve the possibility of conveying the disease and establishing new infections.

Although several descriptions have been written in attempting to illustrate the manner in which the root-rot organism spreads through the soil and to define its relationship to its food supply, none of them, in the opinion of the writers, have succeeded in giving a complete or an entirely accurate account. This seems somewhat surprising in view of the fact that the mycological literature includes descriptions of several well-known fungi whose subterranean behavior is in many ways similar to that of *Phymatotrichum omnivorum*. One of the closest analogies is the destructive mushroom root-rot fungus (*Armillaria mellea*). This fungus leads a saprophytic existence on stumps and roots of dead trees, but under favorable conditions becomes a serious parasite. Although it frequently develops sporophores which produce basidiospores in enormous numbers, it also relies to a large extent on rhizomorphs for its dissemination. Massee (10) speaks of these cordlike rhizomorphs as being in reality elongate sclerotia which radiate in every direction in the soil, growing by the tips only. Heald (4, p. 798, 801) relates that they have an "apical growing region by the activity of which they advance through the soil or over the surface of the host. * * * The rhizomorphs spread in the soil from their original bases (on dead roots or stumps) to adjacent living hosts," etc. Although the strands or rhizomorphs of the cotton root-rot fungus are not so large and probably not so resistant to unfavorable conditions as those of the mushroom root-rot fungus, it is evident that they function in much the same manner. The behavior of the *Phymatotrichum* strands in cultures indicates that they are capable sometimes of developing a beadlike string of sclerotia throughout their length.

A study of the distribution of the root-rot mycelium through the soil in a favorable location in California furnished fairly definite proof

⁵ McNAMARA, H. C., and HOOTON, D. R. Op. cit.

that it is able to draw large quantities of nutritive material from dead root tissues in building up conidial mats or that it is able to accumulate in the soil for long periods. Mats appeared in enormous numbers in trenches at a considerable distance from any living host plants and where only dead tissues were in evidence in the soil. It was also apparent from the behavior in this location that the organism makes more rapid headway where plant roots or other food materials are abundant than where there is a scarcity of this material. Laboratory experiments have shown that the mycelium apparently does not derive suitable nutrients for sustaining growth from soil that contains no organic matter other than that which has been entirely broken down to the condition of humus, woods mold, muck, and peat.

All of these materials have been tried as food media in cultures without obtaining a satisfactory growth of mycelium. In the above-mentioned California location, where there is an infected area entirely isolated, there was evidence that the fungus had crossed bare roadways on two sides of an infected area and extended into new fields with the assistance of dead tree roots. In a field where cotton had been planted for about seven successive years, no infection was observed until 1927, when three plants were killed and about 60 neighboring plants showed infected roots. From this infection dead roots of an almond tree, covered with mycelial strands, were traced from the cotton to the opposite side of a roadway, though the trees were no longer in existence, as they succumbed to the disease and had been dug a year or two before. In another case the conducting tree roots had reached a more advanced stage of decay, but the disease had crossed a roadway to infect a date orchard, where no injuries to the palms above the ground were apparent.

The mycelium, however, had apparently received sufficient nourishment from the dead tree roots and from dead or living date roots to keep it alive and active, but the rate of progress had obviously been much slower where the quantities of deciduous tree roots diminished and the organism was made to rely more largely on date roots for nutrients.

As in the case of *Armillaria mellea*, the strands of *Phymatotrichum* are thus visualized radiating in all directions from a food-supply base, such as a plant root, and ramifying through the soil. When a susceptible plant root or other suitable food material, either of living or dead roots, is met by the advance strands, the mycelium accumulates around these as a new base, and as they afford a supply of food and provide for continued growth of the fungus, they are followed for long distances. In the case of a closely planted crop, such as alfalfa, there seems to be a tendency for the attack on the roots to be confined to the upper 2 or 3 feet, but on tree roots the strands have been found at a depth of 6 feet. Laboratory experiments have shown that the strands may extend for at least 34 inches through a soil that is inert and entirely devoid of nutrient material. In most soils a new supply of suitable food would probably be encountered in that distance to assist in further extension of the mycelium.

The rate at which the disease progresses by this underground method appears to be variable. On one area cropped to cotton continuously for many years it was noted that the disease in one isolated spot of infection advanced into uninfected territory a distance

of 53 feet in eight years. In an alfalfa field it was observed that a single circular spot of infection increased its diameter more than 60 feet in three years.

SUMMARY

Pure cultures of the cotton root-rot fungus *Phymatotrichum omnivorum* (Shear) Duggar, have been grown for periods of months on dead tissues of cotton and many other plants, and many successful inoculations of cotton plants have been obtained under partially controlled conditions by using these cultures. Hence it is plain that the root-rot organism is capable of living on dead plant tissues in the soil and can be communicated from these to susceptible plants. The facility with which plants can be inoculated from infected plant tissues, whether alive or dead, indicates that the transportation of such infected plant material may play a part in the dissemination of the disease.

The extensive distribution of the root-rot mycelium on the roots of certain monocotyledonous plants, such as date palm, Johnson grass, and Bermuda grass and the ability of the fungus to grow continuously for several months on the roots of these plants in cultures, indicate that the organism may be able to subsist on host plants of this type which would serve as carriers for the infection.

Certain plant roots are apparently better suited than others for nourishment of the root-rot fungus. On steam-cooked food, or alcohol-disinfected roots of some of the monocotyledonous plants such as date palm, grasses, corn, and barley, the fungus can be maintained for long periods, but the mycelium grows very slowly.

All attempts by the writers to culture the fungus on the fresh roots of citrus species have been unsuccessful, and from the manner in which the hyphae are inhibited in approaching these roots, it is apparent that the roots contain some material which interferes with the growth of the mycelium.

Cooked roots of some of the most susceptible plants, as cotton, Malva, and peach, provide a more favorable medium for the growth of the fungus than roots of some of those less susceptible, as cottonwood, tamarisk, jujube, and peppertree.

Live root-rot mycelium on decayed tree roots at a depth of 6 feet in the soil of some infected areas and the extensive development of conidial mats at this depth, in trenches, provide evidence that the organism is able to function throughout a large volume of soil and to considerable depths. For this reason difficulties of controlling the fungus by tillage or other methods which affect only the surface layer of soil are to be expected.

Barriers of oil mixed with soil, placed in narrow trenches about 3 feet deep, in front of the advancing line of infection were effective in checking the spread of the disease in alfalfa fields. Sheets of galvanized iron 26 to 36 inches wide, set on edge in trenches prepared in a similar manner, were equally effective.

A clean fallow maintained on two plots at Sacaton, Ariz., for two years had no effect in reducing the extent of the infection when the area was replanted to cotton. On an adjacent plot of the same size, fallowed for one year after a winter crop of peas had been turned under, no reduction in infection resulted from the treatment. When replanted to cotton after fallowing, the infected areas in all these plots were in nearly the same locations and maintained somewhat similar shapes and sizes as before.

The large pseudosclerotial formations developed in artificial cultures are more elaborate structures than the small wartlike aggregates of hyphae found on infected roots in the soil, which also are considered as pseudosclerotia. For their most extensive development, suitable nutrients and an abundant moisture supply are required. Although these large mycelial masses are capable of preserving the life of the fungus for long periods under laboratory conditions, favorable conditions are required for them to remain viable, and they have not been found under natural conditions.

A true type of sclerotium formed from mycelial strands extending through sand and soil in pure cultures was discovered in September, 1928. These sclerotia appeared in great numbers in fruit jars and other containers in which sterile sand and dead cotton-plant tissues were present. Their production apparently is influenced by temperature conditions, but when fully developed they are capable of prompt germination, and show a vigorous growth of new mycelium under suitable temperature and moisture conditions.

The sclerotia are small round or oval tuberlike structures, attaining a diameter of 1 to 2 millimeters, of rather firm texture, showing in section rather large, closely packed cells, with one or more surface layers of smaller and harder cells of very irregular form. The structure of the sclerotia indicates that they are simple storage bodies that may serve for preserving the organism during periods of inactivity. They apparently are not injured by widely fluctuating temperature conditions, or by submergence in water for long periods, but do not seem to withstand desiccation in the air.

The hyphae which develop from the germination of the sclerotia consist of the large, Rhizoctonia-like cells, which characterize a new growth of mycelium in this fungus. These in a short time begin the formation of strands which spread over the substratum in a characteristic manner.

The sclerotia provide a convenient means of propagating the fungus in the laboratory, since under the ordinary conditions of culture they give assurance of immediate and rapid mycelial growth, and contaminations are easily excluded because the sclerotia permit the use of antiseptic solutions on their surface tissues without injuring their viability.

Although the sclerotia have not as yet been found in nature, they doubtless occur under certain conditions, and may play an important rôle in maintaining the fungus through the winter. Also they may furnish an explanation of disappointing results with control measures in various places.

The indications that sclerotia may be formed in great numbers in the soil at certain periods of the year make it unsafe to assume that the disease can not be transmitted in soil from infected areas.

A method was devised for studying the elongation of the strands by establishing a pure culture of the fungus on small sections of dead cotton root in one end of long glass tubes containing sterile moist sand and measuring the daily growth of the strands. By this simple method the fact was established that the mycelial strands are capable of extending long distances from their food supply. Growth in one tube was continued for 46 days, the mycelium being nourished only by a small piece of dead tissue of a cotton plant.

In many of the tubes the strands of the fungus grew through a column of acid-treated sand a distance of 2 feet or more from the food supply. In one of the tubes a vigorous growth of the fungus was continued for 34 inches, when the end of the tube was reached.

In several of the tube cultures the plant tissues on which the fungus had been introduced were removed after a few days, and this was found to have little effect in retarding the growth of the strands. It appeared from these experiments that the fungus is capable of translocating nutrients from the older portions of its system for producing new apical growth.

The opportunity which the sand-tube cultures afford for observing and measuring the elongation of the strands renders this method applicable for studying the effect of various environmental factors on the growth of the fungus.

The presence of secondary organisms in cultures of the root-rot fungus retarded the growth of the mycelium and in some cases checked it completely. Under natural conditions the effects of other soil organisms upon the fungus may determine the distribution of the disease.

The fungus grows through the soil by means of its mycelial strands for considerable distances, and thus is capable of moving from one food supply to another. Though the spread of the disease into new areas by this means is apparently slow, more rapid progress may be made where the soil is pervaded with roots, so that a more continuous food supply is available.

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CORRELATION STUDIES WITH INBRED AND CROSSBRED STRAINS OF MAIZE¹

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INTRODUCTION

The final test of every inbred line of maize is the ability of its crosses to produce large yields of sound corn. An extensive program of selection within selfed lines involves much labor and expense. Good lines could be produced more cheaply, and more rapid progress could be made if it were possible to recognize and discard in the earlier years of selfing those lines that are likely to give unproductive crosses. The studies herein reported were undertaken primarily to determine characters associated with productivity in the hope that these characters might then be used as indexes for selection.

The problem has been developed along three main lines. Coefficients of correlation have been computed (1) among a number of different characters in the same generation (*a*) within inbred lines and (*b*) within F_1 crosses, (2) between characters in the inbred parent lines and the same characters in the crossbred progenies, and (3) between the various characters of the inbred parent lines and the yield of the crossbred progenies. The correlations within lines or crosses (1) are of interest in indicating the characters within the crop itself which are associated with productivity. The parent-progeny correlations (2) indicate the extent to which the parental characters are expressed in the crosses, and (3) they indicate the degree to which characters of the parents influence productivity in the crosses. The parent-progeny correlations, therefore, are the most valuable as guides for selection.

Detailed data are presented on a number of characters of the parent lines and of their F_1 crosses in order to bring out some interesting relations in regard to differences in prepotency among inbred lines of corn. These relations are not brought out by the coefficients of correlation.

REVIEW OF LITERATURE

The extensive studies that have been reported on the relation between various characters and yield in open-pollinated corn need not be considered here. Because of the open-pollinated condition, they have no direct bearing on the present investigations. More recently several investigators have reported on the correlation of characters in inbred lines and their crosses.

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² The writer wishes to acknowledge his indebtedness to Dr. E. W. Lindstrom and F. D. Richey for their kindly criticism and many helpful suggestions during the progress of these investigations, to Prof. G. W. Snedecor and A. E. Brandt for many suggestions in regard to the mathematical phases of the problem, and to A. A. Bryan for very valuable assistance during the selection of the inbred lines and the making of the F_1 crosses.

Kiesselbach (5)³ found a general relation between the productivity of inbred parents and that of their hybrid offspring. Exceptions to this general rule, however, were noted.

Richey (8) found that the tendency of certain strains to produce high-yielding crosses was very noticeable. For example, the mean yield of the seven crosses involving a certain strain exceeded the yield of any single one of the remaining 34 crosses not involving this strain. In later experiments with the same material Richey and Mayer (10) found that some inbred lines were much superior to others in producing high-yielding crossbred combinations.

Kyle and Stoneberg (6) noted that inbred lines having smaller numbers of kernel rows had a greater length of ear per plant, were more resistant to corn smut, had fewer plants with certain heritable deleterious characters, and were generally more vigorous and productive than the lines having larger numbers of kernel rows.

Hayes (2) presented a number of coefficients of correlation to show the inheritance of various characters through different generations of inbreeding. In general, these correlations were positive, as would be expected. The larger coefficients were obtained for length of ear, size of seed, and cob discoloration, although, for some varieties, percentage of smut infection and lodging were rather strongly correlated in the different selfed generations. A number of coefficients of correlation between yield of the inbred lines and various other characters also were given. Yield was found to be strongly correlated with other characters of the inbred lines which are expressions of vigor.

More recently, Nilsson-Leissner (7), in experiments conducted in Minnesota, found that some inbred lines were distinctly superior to others as parents of crosses. He reported the yields of most of the possible combinations among 13 dent inbreds and among 9 flint inbreds. Both among the dents and among the flints certain inbred lines produced higher yielding F_1 crosses. Coefficients of correlation between certain characters in the selfed lines and the same characters in F_1 crosses also were reported. The correlations were positive in every case. Correlations between the yield of the F_1 cross and the mean yield of the two parental lines were 0.1852 ± 0.0580 in the group of dent inbreds, and 0.7434 ± 0.0427 in the group of flint inbreds. Multiple correlations between yields of the F_1 crosses and five characters in the parental lines were 0.6687 in the dents and 0.8240 in the flints.

Jorgenson and Brewbaker (4), in experiments also conducted in Minnesota, obtained data on 10 inbred lines from the dent variety Silver King and on the F_1 crosses between them. Both high and low yielders were found among the crosses from each inbred line. On the basis of the average yield of all of the F_1 crosses in which they were used as parents, some inbred lines appear distinctly superior to others. These investigators also report a number of correlations between various characters in the F_1 crosses and the mean value of the same characters in the two parental lines. Their coefficients of correlation, like those of Nilsson-Leissner (7), are all positive. They calculated a multiple correlation with yield of the F_1 cross as the dependent variable and length of ear, diameter of ear, number of

³ Reference is made by number (*italic*) to "Literature cited," p. 721.

kernel rows per ear, height of plants, and yield in grams per hill of the parent lines as the independent variables. This correlation was 0.6074. The highest simple correlation with yield of the F_1 cross was that of 0.5000 ± 0.0771 with yield of grain in the parents.

MATERIAL

The inbred lines used in these experiments are listed in Tables 1 and 2. These tables show the pedigree number of each line, the variety from which it originated, and a summary of the data on its F_1 crosses. The lines are entered in the tables in the order of their occurrence in the crossing blocks. The crossing-block row numbers are used to designate the lines in other parts of the paper. Most of the inbred lines were produced at Ames, Iowa, during the progress of these investigations. Five inbred lines (Nos. 41, 42, 174, 175, and 176) were obtained from J. R. Holbert, of the Office of Cereal Crops and Diseases, United States Department of Agriculture, Bloomington, Ill., and one inbred line (No. 112) was obtained from E. W. Lindstrom, of the department of genetics, Iowa State College.

Most of the inbred lines listed in Tables 1 and 2 were used both in the correlation studies within inbred lines and in the parent-progeny correlations. There were a few exceptions, however, which are indicated in the tables. In the computation of the coefficients of correlation within inbred lines data were included on 10 lines not used in crosses. Naturally these lines had to be omitted from the parent-progeny correlations. Conversely, in the computation of the correlations within F_1 crosses data were included on the crosses of 12 parent lines for which no data were available on the inbred lines themselves. In the parent-progeny correlations these crosses could be paired only with the one parent for which data were available.

TABLE 1.—Crossing-block row numbers, pedigree numbers, parent varieties, and the means for the different characters in all of the crossbred progeny of each inbred parent line of corn in 1924

Color group and crossing-block row No.	Pedigree No.	Parent variety	Means for the different characters in the crossbred progeny of each inbred line																			Yield per row				
			Number of crosses averaged	Date of—		Shrinkage of harvested ears	Plant height	Number of nodes		Nodes below ear	Plants smutted		Plants erect at harvest		Ears moldy	Suckers per 100 plants	Plants with 2 or more ears	Ears per plant	Ear length	Ear diameter	Ear-shape index (di-amerter × length)		Mean number of kernels per ear	Shelling percentage		
				Tasseling	Silking			Per cent	Ft.		Per plant	Below ear	Per cent	Per cent											Per cent	Per cent
White corn:																							Lbs.			
1 ^b	5-3-1-1	Four-County White	10	18.4	22.0	26.4	6.90	13.4	7.9	59.0	2.5	76.6	11.2	6.5	1.1	1,019	16.2	4.58	0.282	16.0	16.2	4.58	0.282	16.0	84.6	12.07
2	13-1-4-2	do	10	20.6	22.4	25.0	6.60	14.0	8.2	58.3	8.8	39.2	21.2	2.4	1.3	989	18.9	4.54	0.242	14.8	18.9	4.54	0.242	14.8	86.5	13.44
3	20-5-1-2	do	10	16.0	19.2	22.2	6.95	12.2	6.8	55.5	1.8	77.6	18.7	3.1	1.8	1,009	16.5	4.38	0.261	14.2	16.5	4.38	0.261	14.2	85.3	11.19
4	27-1-2-4	do	10	17.6	22.0	24.5	7.00	12.8	7.8	60.5	6.5	60.7	18.8	2.7	1.7	1,004	18.0	4.55	0.254	14.4	18.0	4.55	0.254	14.4	86.2	13.02
5	36-1-4-2	do	10	17.1	19.2	24.2	6.90	12.8	7.4	57.9	5.0	34.2	17.8	2.5	1.4	1,980	15.9	4.64	0.263	14.2	15.9	4.64	0.263	14.2	87.7	12.53
6	63-4-3-5	do	10	18.1	20.4	23.2	6.95	13.3	7.8	58.9	2.1	82.6	14.8	4.8	1.4	1,007	17.3	4.60	0.267	14.5	17.3	4.60	0.267	14.5	85.0	12.78
7	82-1-1-5	do	10	17.5	19.4	20.5	7.20	13.0	7.8	60.2	2.1	82.6	14.8	4.8	1.4	1,007	17.3	4.60	0.267	14.5	17.3	4.60	0.267	14.5	85.0	12.78
8	111-4-5-5	Silver King	10	17.1	19.2	21.5	7.20	13.0	7.2	56.5	1.4	35.2	12.2	3.9	3.0	1,028	17.6	4.44	0.262	14.4	17.6	4.44	0.262	14.4	87.4	13.24
9	122-2-3-4	do	10	16.5	19.4	23.4	6.90	12.6	7.2	56.9	1.4	35.2	12.2	3.9	3.0	1,030	17.6	4.44	0.265	14.4	17.6	4.44	0.265	14.4	87.4	13.24
10	10-2-1-1	Four-County White	9	16.4	19.4	23.4	6.90	12.6	7.2	57.1	2.2	55.5	14.3	5.4	1.3	994	16.3	4.61	0.265	14.4	16.3	4.61	0.265	14.4	86.1	11.84
11	16-1-4-1	do	9	16.9	19.5	20.2	6.65	12.6	7.6	57.5	2.2	55.5	14.3	5.4	1.3	994	16.3	4.61	0.265	14.4	16.3	4.61	0.265	14.4	86.1	11.84
12	16-1-4-1	do	9	17.0	19.2	22.3	6.95	12.4	7.6	60.8	3.1	59.0	17.4	8.3	1.6	1,041	16.7	4.62	0.281	14.2	16.7	4.62	0.281	14.2	86.7	12.25
13 ^b	24-3-1-6	do	9	17.0	19.2	22.3	6.95	12.4	7.6	60.8	3.1	59.0	17.4	8.3	1.6	1,041	16.7	4.62	0.281	14.2	16.7	4.62	0.281	14.2	86.7	12.25
14	31-5-5-2	do	9	15.7	18.6	22.7	7.10	12.7	7.0	55.3	1.9	84.7	9.2	3.1	3	1,022	17.0	4.49	0.273	14.9	17.0	4.49	0.273	14.9	84.3	11.96
15	40-1-2-1	do	9	17.1	20.2	21.3	6.90	13.0	7.7	58.9	2.8	28.2	18.0	4.4	2.0	1,033	17.4	4.62	0.267	14.9	17.4	4.62	0.267	14.9	86.6	13.52
16	74-5-5-4	do	9	16.0	18.1	20.6	7.10	13.0	7.6	58.3	1.1	73.9	14.4	4.8	2.6	1,034	16.4	4.72	0.265	14.3	16.4	4.72	0.265	14.3	86.0	12.69
17	87-3-2-1	do	9	17.2	19.8	24.2	6.80	12.5	7.4	59.0	1.5	80.2	6.4	4.6	1.2	1,004	15.9	4.52	0.285	14.3	15.9	4.52	0.285	14.3	86.0	12.69
18	107-4-4-1	do	9	18.9	20.7	25.2	6.80	13.5	8.0	59.1	8.1	37.9	36.4	6.3	2.5	1,013	17.2	4.53	0.268	16.2	17.2	4.53	0.268	16.2	87.1	12.69
19	115-3-2-3	Silver King	9	17.0	19.9	24.1	6.85	12.8	7.2	56.7	9.2	66.4	27.1	4.4	1.7	1,015	17.8	4.39	0.248	13.6	17.8	4.39	0.248	13.6	85.3	12.09
20 ^b	497-3-4-1	Western Flint	9	24.4	26.8	26.9	7.20	14.4	8.6	59.4	7.5	52.2	16.5	6.9	4.2	1,016	19.5	4.48	0.231	13.4	19.5	4.48	0.231	13.4	83.9	13.40
Mean				17.7	20.2	23.5	6.91	13.0	7.6	58.2	3.6	60.8	16.1	4.3	1.6	1,008	17.2	4.55	0.267	14.6	17.2	4.55	0.267	14.6	86.1	12.66
Early yellow:																										
21	129-4-2-4	C. I. 133	10	17.4	19.6	24.2	7.5	13.9	8.0	57.4	15.1	43.0	27.9	5.5	2.7	0.928	15.9	4.24	0.270	14.1	15.9	4.24	0.270	14.1	83.8	9.10
22	147-3-4-1	do	10	18.0	21.0	24.0	7.2	13.4	8.2	60.9	17.8	68.6	14.8	6.2	3.9	1.036	16.6	4.36	0.264	14.4	16.6	4.36	0.264	14.4	85.8	11.50
23	280-3-6-1	C. I. 204	10	20.3	24.6	27.4	7.4	13.1	7.8	59.2	1.8	63.9	10.7	7.2	1.6	1.032	17.0	4.76	0.279	16.8	17.0	4.76	0.279	16.8	84.9	12.41
25	420-2-7-6	Oosterland's Reid	10	20.8	23.5	27.3	7.2	13.4	8.0	59.2	1.7	88.0	8.6	6.4	2.3	1.053	18.6	4.61	0.250	14.5	18.6	4.61	0.250	14.5	85.3	14.05

TABLE 1.—*Crossing-block row numbers, pedigree numbers, parent varieties, and the means for the different characters in all of the crossbred progeny of each inbred parent line of corn in 1924—Continued*

Color group and crossing-block row No.	Pedigree No.	Parent variety	Means for the different characters in the crossbred progeny of each inbred line																								
			Number of crosses averaged		Date of—		Shrinkage of harvested ears	Plant height	Number of nodes		Nodes below ear		Plants sinited		Plants erect at harvest	Ears moldy	Suckers per 100 plants	Plants with 2 or more ears	Ears per plant	Ear length	Ear diameter	Ear-shape index (diameter × length)	Mean number of kernels per ear	Shelling percentage	Yield per row		
			Tasseling	Silking	Per cent	Fed			Per plant	Below ear	Per cent	Per cent	Per cent	Per cent												Per cent	Per cent
Later yellow:																											
68	356-5-3-3	Black's Reid	10	23.2	26.8	25.9	7.75	13.9	8.0	37.4	5.2	87.1	7.6	9.7	1.049	21.0	4.61	0.222	15.6	18.4	4.94	267	18.4	85.1	14.92	13.41	
69	363-4-2-5	Proudfit's Reid	10	27.0	28.4	23.3	8.00	14.9	8.6	37.4	4.5	37.5	15.6	27.0	1.086	19.5	4.98	0.256	16.5	18.8	4.98	254	16.5	87.0	15.41	16.19	
70	364-5-4-3	do.	10	23.1	25.2	25.5	7.80	14.6	8.4	37.6	7.3	80.8	17.6	31.7	1.064	19.4	4.80	0.251	16.8	18.8	4.80	251	16.8	88.2	15.70	15.70	
71	377-3-1-4	do.	29	26.1	28.3	29.4	8.64	15.5	9.0	58.4	8.7	67.7	16.3	11.4	1.066	18.6	4.80	0.261	17.2	17.2	4.80	261	17.2	87.1	15.70	15.70	
72	385-5-6-2	Krizer Bros. Yellow Dent	10	23.8	25.4	27.2	8.20	14.8	8.4	57.0	4.0	75.0	11.5	11.6	1.026	18.8	5.02	0.270	17.8	17.8	5.02	270	17.8	85.8	15.55	15.55	
73	390-3-1-2	do.	9	27.6	30.3	23.0	8.44	14.3	8.2	56.7	8.4	86.4	7.6	9.7	1.009	18.7	4.94	0.267	18.4	18.4	4.94	267	18.4	85.1	14.92	13.41	
74	393-2-6-2	do.	10	27.7	30.8	25.6	8.15	14.5	8.8	60.2	8.2	68.4	13.8	12.0	1.079	20.2	4.68	0.234	16.5	16.5	4.68	234	16.5	84.8	15.41	16.19	
75	401-1-2-5	McCulloch's Reid	28	23.3	27.8	22.5	8.11	15.0	9.0	60.6	2.2	81.2	18.2	20.2	1.084	19.0	4.82	0.254	16.4	16.4	4.82	254	16.4	88.1	16.19	16.19	
76	406-3-2-1	do.	10	25.7	28.9	23.8	7.92	14.6	8.6	59.0	7.7	50.1	11.0	5.6	1.016	18.7	4.77	0.258	18.1	18.1	4.77	258	18.1	86.5	15.88	15.88	
77	412-5-4-4	do.	10	27.6	28.9	22.8	8.65	14.8	8.4	57.1	3.2	80.3	17.7	17.5	1.056	20.0	5.04	0.254	17.5	17.5	5.04	254	17.5	86.8	17.41	17.41	
78	456-3-2-1	Walden Yellow Dent	10	25.0	26.0	25.0	8.10	15.2	8.8	57.6	1.4	87.8	8.7	6.2	1.023	20.9	4.56	0.220	15.2	15.2	4.56	220	15.2	85.6	15.71	15.71	
79 ^a	461-2-1-1	do.	10	26.2	30.0	25.5	8.68	14.7	8.0	54.2	8.8	76.4	9.2	27.7	1.161	20.4	4.71	0.233	17.3	17.3	4.71	233	17.3	86.7	15.98	15.98	
80	467-1-4-1	do.	10	23.0	25.5	25.2	7.90	14.8	8.3	56.2	9.9	74.0	11.4	8.8	1.1	19.6	4.66	0.238	15.6	15.6	4.66	238	15.6	87.0	16.08	16.08	
Mean				21.8	26.8	24.2	8.03	14.6	8.3	57.1	3.1	69.7	11.3	14.0	1.4	1.063	19.9	4.75	0.242	16.4	16.4	4.75	242	16.4	86.1	15.73	15.73

^a The dates of tasseling and of silking are recorded as the number of days after June 30.

• Used as the parents of crosses, but no data on the lines themselves were available for use in the correlation studies.

TABLE 2.—Crossing-block row numbers, pedigree numbers, parent varieties, and the means for the different characters in all of the crossbred progeny of each inbred parent line of corn in 1926

Color group and crossing block-row No.	Pedigree No.	Parent variety	Means for the different characters in the cross-bred progeny of each inbred line								
			Crosses averaged	Date of—		Shrinkage of the harvested ears	Plant height	Plants erect at harvest	Ears moldy	Shelling percentage	Yield per row
				Tusseling	Silking						
White corn:			No.		P.ct.	Feet	P.ct.	P.ct.		Lbs.	
101	3-3-3-3-composite	Four-County White	10	25.1	28.4	11.1	6.8	95.0	4.8	86.4	11.08
102	11-4-1-3-composite	do	10	25.1	30.2	12.2	7.6	93.8	2.7	85.3	11.86
103	16-4-3-3-composite	do	10	22.9	27.0	11.7	6.8	91.7	4.3	86.4	11.20
104	29-3-5-4-composite	do	9	24.0	26.4	10.3	6.6	91.9	4.1	85.4	11.19
105	46-5-4-2-composite	do	9	25.8	30.0	11.9	6.9	97.2	2.5	84.1	11.23
106	56-3-3-4-composite	do	10	26.0	29.0	13.6	7.1	89.6	1.4	84.3	11.51
107	63-4-1-6-composite	do	10	24.6	28.1	12.4	7.3	93.3	7	85.5	12.29
108 ^b	67-4-2-1-composite	do									
109	80-1-3-6-composite	do	10	26.1	29.6	14.3	6.9	94.8	3.4	84.6	11.32
110	101-4-5-5-composite	do	10	25.5	28.6	12.7	7.3	94.2	3.0	84.4	12.09
111	128-1-3-2-composite	Silver King	9	24.8	27.5	11.9	7.3	86.1	6.2	84.7	11.42
112 ^c	Lindstrom 7117-composite.	White Flint	9	25.7	29.5	13.7	7.4	86.7	1.6	85.6	12.35
113 ^b	50-5-3-6-composite	Four-County White									
	Mean			25.0	28.6	12.4	7.1	92.3	3.1	85.1	11.60
Yellow corn:											
114	134-3-2-4-composite	C. I. 133	9	27.1	32.4	14.6	7.6	73.4	4.8	83.2	10.06
115 ^b	135-4-5-6-composite	do									
116	153-5-2-2-composite	do	9	26.4	30.4	13.8	7.0	89.0	2.5	85.2	9.84
117	155-2-2-2-composite	Iodent	9	34.4	39.0	17.1	7.8	68.6	4.6	83.0	10.41
118	157-3-1-3-composite	do	9	34.5	38.4	17.7	7.8	74.0	2.1	82.5	10.31
119	161-1-3-3-composite	do	9	34.5	39.0	19.7	8.4	79.2	2.6	80.8	10.68
120	169-4-4-1-composite	do	9	34.4	39.4	13.8	8.0	49.7	3.8	83.0	11.08
121	170-2-3-2-composite	do	42	33.2	37.8	15.5	7.9	80.2	2.8	83.8	10.38
122 ^b	183-4-5-2-composite	do									
123	188-1-4-1-composite	do	9	32.2	38.0	16.4	7.9	82.1	5.2	84.6	10.35
124	197-1-2-6-composite	do	9	32.6	35.7	17.3	8.1	85.0	2.5	83.5	12.30
125	207-2-5-2-composite	do	8	32.8	37.0	18.6	7.9	75.7	2.9	83.6	10.90
126	215-2-5-1-composite	do	9	33.4	38.1	15.9	8.4	80.1	1.3	84.2	11.85
127 ^b	219-3-1-5-composite	do									
128	224-2-2-1-composite	do	9	34.6	38.4	15.2	8.2	86.6	1.8	84.0	11.72
129	234-2-3-1-composite	do	9	34.2	38.1	16.2	8.2	75.0	3.1	83.7	11.16
130	254-3-6-1-composite	do	9	36.5	40.7	20.1	8.3	66.3	6.3	83.8	10.91
131 ^b	262-3-3-2-composite	do									
132	267-3-5-2-composite	do	9	34.6	39.2	15.8	8.0	77.6	1.9	83.4	11.13
133	275-3-5-1-composite	C. I. 204	9	34.9	39.4	17.6	7.8	72.1	3.9	84.1	9.85
134 ^b	278-3-4-1-composite	do									
135	289-4-3-5-composite	Lancaster Surecrop	9	32.0	37.1	16.1	7.8	63.3	4.7	84.6	12.43
136	291-1-6-1-composite	do	9	33.6	38.4	17.3	8.0	45.3	1.3	83.2	11.65
137 ^b	307-2-4-4-composite	do									
139	315-2-4-3-composite	do	9	32.4	37.6	18.7	8.0	50.2	2.7	82.3	11.39
140	317-3-1-2-composite	do	41	34.2	39.4	19.8	8.4	62.4	4.2	81.6	11.46
141	324-2-2-1-composite	do	9	32.4	37.7	19.6	7.8	61.5	9.5	82.5	11.17
142	331-3-1-7-composite	do	9	31.0	35.8	19.5	7.9	49.0	3.5	80.8	11.76
143	345-2-1-5-composite	Black's Reid	43	33.8	38.4	15.2	8.4	75.1	5.5	82.7	10.56
144	348-3-1-5-composite	do	9	33.8	38.7	16.2	7.9	61.4	1.4	82.0	10.98
145 ^b	349-5-1-6-composite	do									
146	351-4-5-5-composite	do	9	33.7	38.1	17.1	8.2	71.4	3.7	84.8	12.27
147	353-5-1-1-composite	do	9	34.6	39.3	19.3	8.0	82.1	3.2	85.4	9.79
148 ^b	358-2-6-2-composite	Proudfit's Reid									
149	365-4-3-1-composite	do	9	32.8	38.6	19.0	8.4	76.0	1.9	84.4	10.39
150	370-1-1-1-composite	do	43	33.6	38.9	18.4	7.6	68.6	3.1	84.8	10.57
151	389-5-2-1-composite	Krizer Bros. Yellow Dent.	7	34.8	38.2	19.7	7.8	41.4	1.8	85.1	10.16
152 ^b	391-5-5-1-composite	do									
153	394-4-2-1-composite	do	43	32.0	36.1	17.6	7.6	82.7	2.5	84.1	10.79
154	397-1-2-1-composite	do	9	32.7	37.1	19.7	7.6	64.8	3.0	82.5	11.39
155	398-1-2-2-composite	McCulloch's Reid	9	33.4	37.7	17.6	8.2	78.5	6.9	83.4	11.19
156	399-1-1-6-composite	do	9	31.6	36.1	14.5	8.0	78.6	3.3	84.6	10.49
157	411-3-3-3-composite	do	43	33.0	38.8	19.6	8.1	59.0	4.2	83.5	10.43

^a The dates of tasseling and of silking are recorded as the number of days after June 30.^b Used in the correlation studies within inbred lines but not as the parents of crosses.

TABLE 2.—*Crossing-block row numbers, pedigree numbers, parent varieties, and the means for the different characters in all of the crossbred progeny of each inbred parent line of corn in 1926—Continued*

Color group and crossing block-row No.	Pedigree No.	Parent variety	Means for the different characters in the crossbred progeny of each inbred line								
			Crosses averaged	Date of— ^a		Shrinkage of the harvested ears	Plant height	Plants erect at harvest	Ears moldy	Shelling percentage	Yield per row
				Tasseling	Silking						
Yellow corn:			No.			P.ct.	Feet	P.ct.	P.ct.		Lbs.
158	415-5-4-4-composite	McCulloch's Reid	9	34.3	38.6	19.3	8.0	71.1	5.0	83.5	10.39
159	418-2-6-1-composite	Osterland's Reid	9	30.7	37.8	14.8	7.2	87.5	9.8	82.0	8.65
160	419-2-2-4-composite	do	43	32.0	36.0	16.5	8.1	75.7	5.5	83.4	10.92
161	420-2-7-5-composite	do	9	30.6	36.2	21.2	7.7	87.6	6.0	83.5	11.09
162	433-2-3-1-composite	Clark Yellow Dent	9	35.0	39.7	17.6	8.2	43.5	3.9	81.6	9.63
163 ^b	440-1-3-7-composite	do									
164	447-5-1-8-composite	do	9	27.5	32.9	12.3	7.3	86.1	4.3	81.8	10.30
165	451-1-5-1-composite	Walden Dent	9	33.4	38.6	20.2	7.8	75.4	5.2	84.7	9.75
166	460-4-1-5-composite	do	9	33.8	38.6	19.0	8.2	69.2	3.2	82.9	10.30
167	461-2-1-4-composite	do	9	38.4	43.0	20.3	9.0	58.3	2.0	84.2	10.28
168	465-3-1-4-composite	do	43	32.8	37.1	13.6	7.8	77.0	1.8	83.4	10.41
169	467-1-4-4-composite	do	9	31.5	36.9	20.7	8.0	71.5	3.5	83.6	10.92
170	477-4-2-2-composite	Argentine Flint	9	32.8	39.6	16.5	7.8	84.7	3.4	85.5	9.01
171	483-5-4-4-composite	do	43	33.2	38.8	23.1	7.8	51.1	3.0	83.5	10.40
172	487-5-1-2-composite	do	9	31.8	36.4	17.8	7.8	64.4	1.9	84.9	10.27
173	493-3-1-1-composite	do	9	35.0	42.2	22.5	7.6	55.7	1.1	81.9	9.07
174 ^c	Holbert's A-1-1-2-R-1-2-3-7-1	Funk Bros. 176 A	9	33.6	38.1	18.4	7.8	68.6	2.3	82.7	10.81
175 ^c	Holbert's B-1-1-3-R-10-1-12-14	do	8	37.0	41.6	19.6	8.3	65.4	5.2	83.8	9.89
176 ^c	Holbert's G-8-8-1-B-2-2	Griffin's Reid	9	32.8	36.8	17.2	8.4	66.9	2.3	83.3	9.88
	Mean			33.1	37.9	17.7	8.0	70.2	3.6	83.4	10.65

^a The dates of tasseling and of silking are recorded as the number of days after June 30.^b Used in the correlation studies within inbred lines but not as the parents of crosses.^c Used as the parents of crosses, but no data on the lines themselves were available for use in the correlation studies.

The F_1 crosses were made in 1925 and 1926. Those made in 1925 were compared for yield in 1926, and those made in 1926 were compared for yield in 1927. The lines developed at Ames had been selfed for three generations prior to crossing in 1925, and for four generations prior to crossing in 1926. Lines Nos. 41 and 176, received from Doctor Holbert, had been inbred for 5 generations, No. 42 for 7 generations, and Nos. 174 and 175 for 8 generations. Line No. 112, from Doctor Lindstrom, had been inbred for 2 generations when crossed.

The varieties represented in the experiments and the number of inbred lines originating from each are shown in Table 3. In all, 140 lines from 18 varieties were used as the parents of crosses and 142 lines from 14 varieties in the correlation studies within inbred lines.

TABLE 3.—Numbers of inbred lines from different varieties of corn used in the experiments

Parent variety	Number of inbred lines			
	Used as the parents of crosses in the year stated			Included in the correlations
	1925	1926	Total	
Four-County White	15	0	24	24
Silver King	3	1	4	4
C. I. 133	4	2	6	7
Iodent	18	13	31	34
C. I. 204	3	1	4	5
Lancaster Surecrop	4	6	10	11
Black's Reid Yellow Dent	3	4	7	8
Proudfit's Reid Yellow Dent	3	2	5	6
Krizer Bros. Yellow Dent	3	3	6	7
McCulloch's Reid Yellow Dent	3	4	7	7
Osterland's Reid Yellow Dent	4	3	7	7
Clark Yellow Dent	4	2	6	7
Walden Dent	3	5	8	7
Argentine Flint	4	4	8	8
Western Flint	1	0	1	0
White Flint	0	1	1	0
Funk Bros. 176 A	2	2	4	0
Griffin's Reid Yellow Dent	0	1	1	0
Total	77	63	140	142

EXPERIMENTAL METHODS

Throughout the selection of the inbred lines extreme care has been exercised to prevent accidental outcrossing. All of the self-pollinations since the experiments were started have been made by the bottle method already described (3). Very little outcrossing has occurred. For example, in 1926 less than 0.4 per cent of outcrossed plants occurred in the selfing plots. At this time the lines had been inbred for four generations, and outcrossed plants could be distinguished readily by their greater size and vigor.

MAKING THE F_1 CROSSES

In comparing the inbred lines as parents an attempt was made to use each line in at least 10 crosses. All of the crosses were made in a special group of rows called the crossing block. Several different methods were followed. In 1925 each row in the crossing block was from the seed of an individual ear. In 1926 seed from three to five selfed ears was mixed to represent each line.

The 80 inbred lines in the 1925 crossing block (Nos. 1-80, Table 1) were divided into three groups. The first group, Nos. 1 to 20, were from varieties of white corn; the second group, Nos. 21 to 40, were from early varieties of yellow corn; and the third group, Nos. 41 to 80, were from the later varieties of yellow corn. Lines 8, 23, and 44 were weak and undesirable and therefore were discarded. This left 19 lines in each of the first two groups and 39 in the third group.

Within the first group, each of the lines 1 to 10, inclusive (except 8), was crossed with each of the lines 11 to 20, inclusive, resulting in 90 different combinations. Similarly, in the second group, lines 21 to 30, inclusive (except 23), were crossed with lines 31 to 40, inclusive. This also gave 90 different combinations. In both groups the crosses were made reciprocally, and the seed from reciprocal crosses was

mixed for use in the yield experiments. A slightly different procedure was followed in the third group. Here, 10 of the 39 lines were selected as sires, and an attempt was made to cross each sire with each of the remaining 29 lines. Of the 290 possible combinations, 281, or all but 9 combinations, were obtained. No reciprocal crosses were made in this group.

The 1926 crossing block contained 76 inbred lines (Nos. 101 to 176, Table 2). Lines 101 to 113, inclusive, were from varieties of white corn. Two of these were discarded, the 11 best-appearing lines being selected for crossing. An attempt was made to obtain all possible combinations among these 11 lines. Of the 55 possible combinations, 53 were obtained. Seed of reciprocals was mixed for use in the yield experiments. Lines 114 to 176, inclusive, were from yellow corn. For various reasons 10 of these were discarded, leaving 53 lines. Of these, 10 were selected as sires and were crossed with each of the other 43 lines. Reciprocal crosses were not made in this group. Later in the season, after the crosses had been made, one of the lines used as a sire developed undesirable characteristics and all crosses with it were discarded. All but 4 of the 387 possible combinations (9 sires with each of 43 lines) were obtained.

In making all crosses, pollen from 12 to 15 plants of the row used as the male parent was mixed together and three to six ears were pollinated in each row used as a female parent. This tended to avoid the effect of individual plant variation. In the three groups of lines where reciprocal crosses were made and the seed mixed, therefore, from 12 to 20 plants in each of the parental lines were represented in the cross. In the two groups where reciprocal crosses were not made, 12 to 15 plants of the male parent and 3 to 6 plants of the female parent were represented in the cross.

The technic employed in making the crosses was similar to that described by Coulter (1). A half-ounce bottle was used to hold the pollen, instead of a thistle tube. The top of the bottle was fitted with a 2-hole rubber stopper. Two pieces of glass tubing were inserted, as for an ordinary wash bottle. The pollen then was blown onto the silks. In this way it was easy to make 50 to 60 cross pollinations with one collection of pollen.

YIELD EXPERIMENTS

All data on the inbred parent lines used in the correlation studies were obtained in a special yield experiment in 1926. At this time the lines had been selfed for four generations. The seed used to represent a line of the 1925 crossing block was a mixture from several selfed progeny ears of the ear used in that crossing block. The seed used to represent a line of the 1926 crossing block was from the same mixtures as those used to plant that crossing block.

Each line was planted in three distributed 15-hill, 1-row plots. Every tenth plot was planted to the same inbred line to provide a uniform check. Because of a shortage of seed, only three kernels per hill were planted, and the plants were not thinned.

The data on the F_1 crosses used in these studies were obtained from special yield experiments conducted in 1926 and 1927. The three groups of F_1 crosses, white, early yellow, and late yellow, which were made in the 1925 crossing block were compared in the 1926 experiments, and the two groups of F_1 crosses, white and yellow,

which were made in the 1926 crossing block were compared in the 1927 experiments. In each experiment six distributed 1-row plots, each 15 hills long, were planted with each kind of corn. In order to obtain more uniform stands, four kernels per hill were planted and later the plants were thinned to three per hill. In the 1926 experiments, every tenth plot was planted to a uniform check. No check plots were planted in the 1927 experiments.

All yields are reported as pounds of air-dry shelled corn per row. The percentage of moisture in the comparison of inbred lines was determined by drying the entire yield from each plot. In the 1926 comparison of crosses the percentage of moisture was determined from a shrinkage sample of 15 random ears taken from each plot. In the 1927 experiments the entire yield from two of the six replications of each kind of corn was dried. The average moisture content of the shrinkage samples after they had become air dry was 5.57 per cent for the experiments conducted in 1926, 5.69 for the comparison of white crosses in 1927, and 7.19 per cent for the comparison of yellow crosses in 1927. The yields in pounds per row may be converted to acre yields in bushels of grain containing 15 per cent of moisture by multiplying by the following factors:

For all of the 1926 experiments	5. 191
For the white crosses, 1927.....	5. 184
For the yellow crosses, 1927.....	5. 102

In both the 1926 and the 1927 yield experiments the six plots of each kind of corn were distributed at random over the field. In the 1926 experiments, however, the first replication was planted in the order of the pedigree numbers of one inbred parent and the second replication was planted in the order of the pedigree numbers of the other parent. The four packets of seed of each kind of corn for the remaining four replications then were put together in a churn and thoroughly mixed. They then were drawn at random from the churn and planted in that order. In 1927 six packets of seed were made up of each kind of corn, one packet for each replication. The packets for each replication, however, were mixed separately, so that there was random distribution within each replication, but the different replications were kept separate and distinct.

YIELD COMPUTATIONS

The data were recorded on punch cards which then were used with mechanical sorting and tabulating machines. In this way no more effort was required to collect the data from the various plots of each kind of corn with a random distribution than would have been required with a systematic distribution.

Yields were adjusted for variations in soil productivity and in stand. Adjustments for soil heterogeneity were made according to the regression of the individual rows on a 5-row moving average, as suggested by Richey (9). Adjustments for stand were made according to the regression of yield on stand. The essentially different feature of the process used was that these two adjustments were made on the basis of one multiple regression equation which took account of variation in both soil and stand. The various correlations among stand, the 5-row moving average, and the deviation of the yield of each plot from the mean yield of all plots of the same kind of

corn first were calculated. From these correlations a multiple regression equation of the form

$$\bar{D} = \beta_{DS} \frac{\sigma_D}{\sigma_S} S + \beta_{DA} \frac{\sigma_D}{\sigma_A} A$$

was determined. In this \bar{D} represents the predicted and D the observed deviation in yield of any plot from the mean yield of all plots of the same kind of corn, S represents the deviation in stand of any plot from the mean stand of the experiment, A , the moving average value, and β indicates the partial regression coefficients. Actually only the mean yields of the different kinds of corn were adjusted. Then \bar{D} represents the correction term to be applied to the mean S , the mean deviation in stand of all plots of the same kind of corn, and A their mean moving average value.

A general standard deviation was calculated for each experiment. The formula used was the usual one of

$$\sigma_E = \sqrt{\frac{\sum d^2}{N}}$$

in which d is the deviation of each plot from the mean of all plots of the same kind of corn. The standard deviation of the difference between any two mean yields was then calculated according to the formula suggested by Richey (9) as follows:

$$\frac{2s \sigma_E^2 (1 - R^2)}{(s-1)(n-1)}$$

in which s is the number of plots used in computing the moving average, n is the number of replications, and R is the multiple correlation of stand and moving average with yield.

COLLECTING DATA ON CHARACTERS STUDIED

A list of all of the characters treated as variables in the correlation studies is given in Table 4. The symbols used throughout to designate the respective variables are shown at the left of the table. Table 4 also shows the units of measurement and least measurements used in taking the data on the variables and the class intervals used in calculating the coefficients of correlations. In general, the size of the class interval was arranged so as to give 10 classes.

Data on the characters studied were taken on each replication of the yield experiments, except that the date of tasseling, date of silking, plant height, nodes per plant, and nodes below ear were determined on only two replications of the F_1 crosses. The final values used in the correlation tables were the means of the values determined for the different replications.

The date of tasseling and date of silking are based on the date on which 10 plants in the row (approximately one-fourth of the plants) tasseled or silked. The dates are recorded as the number of days after June 30. A plant was counted as tasseled as soon as anthers appeared.

TABLE 4.—Variables, units of measurements, and least measurements used in taking the data, and the class intervals used in calculating the coefficients of correlation

Symbol	Variable	Units of measurement and least measurements	Class intervals	
			Inbred	F ₁ crosses
A	Date of tasseling	1 day	1 day	$\frac{3}{8}$ day
B	Date of silking	do	do	Do
C	Shrinkage of the harvested ears	1 per cent	2.3 per cent	$\frac{3}{8}$ per cent.
D	Chlorophyll	1 grade	0.3 grade	0.8 node.
E	Plant height	0.5 foot	0.5 foot	0.5 foot.
F	Nodes per plant	Actual number	0.5 node	0.8 node.
G	Nodes below ear	do	0.3 node	0.5 node.
H	Percentage of nodes below ear	1 per cent	2.2 per cent	2.2 per cent.
I	Plants smutted	do	5.1 per cent	5.1 per cent.
J	Plants erect at harvest	do	11 per cent	11 per cent.
K	Ears moldy	do	8.2 per cent	6 per cent.
L	Suckers per 100 plants	Actual number	7.2 suckers	7.2 suckers.
M	Plants with 2 or more ears	1 per cent	9 per cent	2.5 per cent.
N	Ears per plant	Actual number	0.09 ear	0.09 ear.
O	Ear length	0.1 cm.	0.9 cm	0.1 cm.
P	Ear diameter	do	0.216 cm	0.15 cm.
Q	Ear-shape index (diameter÷length)	0.001	0.025	0.020.
R	Kernel rows per ear	Actual number	0.8 row	1 row.
S	Shelling percentage	1 per cent	2.1 per cent	1.05 per cent.
T	Coefficient of variability of number of kernel rows.	0.1 per cent	1.6 per cent	
X	Yield	0.2 pound	0.7 pound	0.7 pound.
Y	Mean yield of crosses	0.01 pound	0.45	

Data on chlorophyll grade were taken on the inbred lines only. Five arbitrary color grades were established. These grades were numbered 1 to 5, inclusive, grade 1 being the darkest color and grade 5 the lightest. Each replication of the inbred lines was graded. The average of the grades given the three replications was taken to represent the line.

Plant height was determined by measuring several representative plants in the row. The mean of these measurements then was computed.

Nodes per plant were the means of the numbers of nodes above the surface of the ground for the first 10 plants in the row. Nodes below ear were determined in a similar manner. When there were two ears the number of nodes was determined to the upper ear. The percentage of nodes below the ear was computed from these data.

Plants smutted, plants erect at harvest, ears moldy, suckers per 100 plants, plants with two or more ears on the main stalk, and ears per plant are self-explanatory. They were determined from the total counts for all replications.

Data on shrinkage of the harvested ears, ear length, ear diameter, ear-shape index, number of kernel rows per ear, and shelling percentage were obtained from the drying samples taken from each plot at harvest. These data were taken only on the inbred lines and on the F₁ crosses compared in 1926.

The coefficient of variability for kernel rows per ear was determined for the inbred lines only.

COMPUTATION OF COEFFICIENTS OF CORRELATION

All of the coefficients of correlation of the zero order, the partial correlations, and the multiple correlations were calculated according to the methods suggested by Wallace and Snedecor (11). The computations were carried to two more places than those reported.

The class intervals shown in Table 4 were used in calculating all of the coefficients of correlation except where otherwise stated. Shepard's correction was not used to correct for the fact that the data were coded.

ADJUSTING FOR HETEROGENEITY OF DATA

Inbred lines from 14 varieties have been included in the present experiments. Some of these varieties differ widely in practically all of the characters studied. In order to group the inbreds from all of these varieties into the same correlation tables, it was necessary to adjust for heterogeneity of material. The method finally adopted was to express the value for any character in an inbred line as a deviation from the mean value of that character in all lines of the variety from which the given line originated. The writer is not entirely satisfied that this is the best method that could have been used. It may be that expressing the value of a character of any line as a percentage of the mean of that character in all of the lines from the same variety would have been more desirable because it would have given more weight to deviations from small means. The method used, however, measures without distortion the average tendency of the variously paired characters toward concomitant variation around the means of the different parent-varietal groups.

The same general method was used with the F_1 crosses grown in 1926, the only ones within which correlations were computed. The parent lines of these crosses had been grouped into three more or less uniform groups (white, early yellow, and late yellow). The mean of each character for each group therefore was determined, and the characters of each F_1 cross were then expressed as deviations from the mean of the group in which that cross occurred.

COEFFICIENTS OF SIMPLE CORRELATION WITHIN INBRED LINES

For convenience in studying the interrelations among the different variables, they have been divided into seven groups of more or less related characters. Except where otherwise stated, the same grouping has been maintained in all of the correlation studies reported. These groups are described as follows:

Group 1.—Characters indicating the relative length of season required to reach maturity, comprising date of tasseling, date of silking, and shrinkage of the harvested ears.

Group 2.—Characters indicating the relative vigor and size of the plants in the different lines and crosses, comprising chlorophyll grade, plant height, nodes per plant, nodes below the ear, and percentage of nodes below the ear.

Group 3.—Characters indicating the relative susceptibility to disease of the different lines and crosses, comprising plants smutted, plants erect at harvest, and ears moldy.

Group 4.—Characters indicating the relative branching habits of the plants, comprising suckers per 100 plants, plants with two or more ears, and ears per plant.

Group 5.—Characters of the harvested ears, comprising ear length, ear diameter, ear-shape index, kernel rows per ear, and shelling percentage.

Group 6.—The coefficient of variability of number of kernel rows, which may be taken as an index of the heterozygosity of the parent inbred lines.

Group 7.—Yield. In some of the parent-progeny correlations the mean yield of all crosses for the parent lines was placed in this group.

The coefficients of simple correlation among the different characters studied in the inbred lines are shown in Table 5. Coefficients which are three or more times their probable errors are considered significant and are printed in boldface type.

Of the 210 coefficients of correlation in Table 5, 65 may be considered significant statistically, as judged by the fact that they are three or more times their probable errors. A summary of the positive and negative relations among the different variables, as indicated by the significant coefficients of correlation in Table 5, is given.

All of the coefficients in Table 5 are between plant or ear characters in the same generation. Their size, therefore, affords an approximate measure of the relative ease with which various combinations of characters could be obtained by selection. It is common knowledge among corn breeders, however, that inbred lines tend to breed true in successive generations. This tendency, which has been shown quantitatively by Hayes (2), gives to these coefficients much of the significance of parent-progeny correlations.

The most interesting correlations shown in Table 5 are those with yield of the inbred line. The highest positive correlation of yield with another variable was that of 0.39 with shelling percentage. The other characters which were significantly and positively correlated with yield were ear length (0.38), ear diameter (0.32), ears per plant (0.31), and plant height (0.20). The characters which were significantly and negatively correlated with yield were shrinkage of the harvested ears (-0.27), date of silking (-0.26), chlorophyll grade (-0.21), and ear-shape index (-0.17). The first two of these negative correlations indicate that late maturity was associated with low yields. Within inbred lines late maturity often is due to a general lack of vigor resulting from the presence of deleterious characters. It is possible that the low yields may have been due to reduced vigor as reflected in late maturity rather than to inherent lateness alone. The negative correlation with ear-shape index indicates that the larger yields comprised relatively long, slender ears. It should be remembered that grade 1 of chlorophyll was the darkest green and grade 5 the lightest. The negative correlation here, therefore, indicates that the dark-green plants were higher yielding.

Date of tasseling and date of silking were significantly and positively correlated with shrinkage of the harvested ears, plant height, nodes per plant, nodes below ear, and plants with two or more ears. The correlation between date of tasseling and shrinkage was 0.37, and that between date of silking and shrinkage was 0.46. This indicates that date of silking was a better index of relative maturity among these inbred lines than was date of tasseling.

The percentage of plants erect at harvest was correlated significantly and negatively with three variables. The highest correlation was that with ears moldy. It naturally would be expected that those lines in which a large number of the plants were down, with many of the ears resting on the ground, would have more moldy ears than lines with erect plants.

Ear-shape index was correlated significantly and positively with shrinkage of the harvested ears, ears moldy, ear diameter, and kernel rows per ear, and was significantly and negatively correlated with ears per plant and ear length. The correlations with ear length, ear diameter, and number of kernel rows naturally would be expected. Those with ears moldy and with shrinkage of the harvested ears indicate that the relatively short, thick ears were more inclined to be moldy and that they shrank the most.

TABLE 5.—Coefficients of simple correlation among various characters within inbred lines of corn

NOTE.—Coefficients of 0.17 are 3 times their P. E. (probable error), those of 0.22 are 4 times their P. E., those of 0.26 are 5 times their P. E., those of 0.31 are 6 times their P. E., those of 0.39 are 8 times their P. E., and those of 0.45 are 10 times their P. E. Coefficients three or more times their P. E. are printed in **Boldface type**

Group No. and symbol	Variable	Date of—		Shrinkage of harvested ears	Chlorophyll grade	Plant height	Number of nodes		Percentage of nodes below ear	Plants erect at harvest	Ears moldy	Suckers per 100 plants	Plants with two or more ears	Ears per plant	Ear length	Ear diameter	Ear shape index	Kernel rows per ear	Shelling percentage	
		Tasseling	Silking				Per plant	Below ear												
X	Yield	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1 A	Date of tasseling	0.15																		
1 B	Date of silking	0.36	0.81																	
1 C	Shrinkage of the harvested ears	0.27	0.03																	
1 D	Chlorophyll grade	0.21	0.37	0.06	0.02															
2 E	Plant height	0.20	0.37	0.38	0.20	0.11														
2 F	Nodes per plant	0.06	0.44	0.34	0.17	0.15	0.43	0.65												
2 G	Nodes below ear	0.16	0.45	0.38	0.17	0.17	0.01	0.08	0.67											
3 H	Percentage of nodes below ear	0.12	0.13	0.15	0.01	0.11	0.19	0.18	0.16	0.04	0.12									
3 I	Plants smutted	0.07	0.06	0.00	0.07	0.09	0.11	0.19	0.16	0.04	0.12									
3 J	Plants set at harvest	0.17	0.18	0.11	0.15	0.12	0.11	0.18	0.16	0.04	0.12									
4 K	Ears moldy	0.16	0.06	0.04	0.04	0.07	0.06	0.12	0.05	0.08	0.12									
4 L	Suckers per 100 plants	0.10	0.12	0.10	0.08	0.02	0.05	0.10	0.16	0.09	0.19									
4 M	Plants with two or more ears	0.11	0.32	0.19	0.02	0.18	0.28	0.35	0.47	0.27	0.09	0.05	0.11	0.34	0.03	0.11	0.09	0.01	0.34	0.79
5 N	Ears per plant	0.31	0.18	0.05	0.10	0.18	0.27	0.28	0.30	0.16	0.14	0.09	0.01	0.03	0.19	0.03	0.11	0.15	0.03	0.79
5 O	Ear length	0.38	0.13	0.21	0.20	0.13	0.03	0.08	0.01	0.04	0.11	0.12	0.06	0.22	0.36	0.29	0.38	0.11	0.36	0.79
5 P	Ear diameter	0.32	0.20	0.23	0.22	0.08	0.07	0.13	0.05	0.03	0.04	0.15	0.08	0.28	0.11	0.06	0.21	0.76	0.31	0.37
5 Q	Kernel rows per ear	0.17	0.03	0.02	0.37	0.06	0.03	0.00	0.02	0.02	0.08	0.02	0.11	0.23	0.25	0.10	0.50	0.37	0.13	0.37
5 R	Shelling percentage	0.04	0.08	0.01	0.10	0.01	0.02	0.13	0.05	0.05	0.02	0.07	0.00	0.00	0.07	0.14	0.30	0.03	0.13	0.37
6 S	Coefficient of variability of number of kernel rows	0.39	0.15	0.26	0.14	0.01	0.19	0.04	0.05	0.03	0.02	0.01	0.07	0.00	0.00	0.21	0.07	0.13	0.12	0.04
6 T		0.12	0.04	0.05	0.07	0.05	0.11	0.10	0.14	0.05	0.02	0.11	0.08	0.03	0.00	0.21	0.07	0.13	0.12	0.04

SUMMARY SHOWING THE SIGNIFICANT POSITIVE AND NEGATIVE RELATIONS AMONG THE DIFFERENT VARIABLES

Symbol	Variable	Characters with which the variable indicated was significantly correlated in the direction stated		Symbol	Variable	Characters with which the variable indicated was significantly correlated in the direction stated		Symbol	Variable	Characters with which the variable indicated was significantly correlated in the direction stated	
		Positive	Negative			Positive	Negative			Positive	Negative
A	Date of tasseling.....	BCDEFGMN.	JP.	H	Percentage of nodes below ear.	GM.	D.	O	Ear length.....	PTX.	BCM.Q.
B	Date of silking.....	ACEFGM.	OP.SX.	I	Plants smutted.....	L.	AFK.	P	Ear diameter.....	OQSRX.	ABLMN.
C	Shrinkage of the harvested ears.	ABFGQ.	OX.	K	Ears moldy.....	Q.	J.	Q	Ear-shape index.....	CKPR.	NOX.
D	Chlorophyll grade.....	ABFGMN.	HMN.X.	L	Suckers per 100 plants.....	IN.	P.	R	Kernel rows per ear.....	PQ.	MN.
E	Plant height.....	ABCEGMN.	S.	M	Plants with two or more ears.	ABEFGHN.	DOPR.	T	Shelling percentage.....	PX.	BE.
F	Nodes per plant.....	ABCEGMN.	J.	N	Ears per plant.....	AEFGLMX.	DPQR.	X	Coefficient of variability of number of kernel rows.	O.	
G	Nodes below ear.....	ABCEFGHMN.							Yield.....	ENOPS.	BCDQ.

COEFFICIENTS OF PARTIAL AND OF MULTIPLE CORRELATION
WITHIN INBRED LINES

Coefficients of partial correlation between yield and other characters of the inbred lines were determined for most of the variables in Groups 1, 2, 3, and 5. The percentage of nodes below the ear was omitted from Group 2, and ear-shape index was omitted from Group 5, as they are both ratios, and partial correlations involving them are likely to be misleading. All of the characters in Group 4 and kernel rows per ear in Group 5 also were omitted.

Partial correlations were computed between each character of each group and the yield of the inbred line, thus eliminating the effect of the variation of the remaining characters of the group. The coefficients of multiple correlation for yield and all of the characters in each group also were computed. These correlations are recorded in Table 6.

TABLE 6.—*Coefficients of partial and of multiple correlation between yield and four groups of the other characters of the inbred lines*

Group No.	Designation of coefficient	Coefficients of correlation	Group No.	Designation of coefficient	Coefficients of correlation
1	<i>YAX.CH</i>	0.11 ± 0.06	3	<i>YIX.JK</i>	-0.08 ± 0.06
	<i>YBX.AC</i>	$-.18 \pm .06$		<i>YJX.IK</i>	$.05 \pm .06$
	<i>YCX.AB</i>	$-.18 \pm .06$		<i>YKX.IJ</i>	$-.15 \pm .06$
	<i>RX.ABC</i>	$.33 \pm .05$		<i>RX.IJK</i>	$.18 \pm .06$
2	<i>YDX.RFG</i>	$-.17 \pm .06$	5	<i>YDX.PS</i>	$.31 \pm .05$
	<i>YRX.DFG</i>	$.17 \pm .06$		<i>YFX.OS</i>	$.17 \pm .06$
	<i>YFX.DEG</i>	$-.09 \pm .06$		<i>YSX.OP</i>	$.31 \pm .05$
	<i>YQX.DEF</i>	$.10 \pm .06$		<i>RX.OP</i>	$.52 \pm .04$
	<i>RX.DRFG</i>	$.30 \pm .05$		<i>RX.ABCDEFGKMOPS</i>	$.69 \pm .03$

The ear characters (Group 5) were most highly correlated with yield. This was to be expected, as these characters represented the physical description of the ears constituting yield itself. The correlation between yield and the group of characters indicating relative disease susceptibility (Group 3) was the lowest. There was but little difference between the multiple correlations for yield with characters of the remaining two groups. The multiple correlation between yield and 12 of the other characters, 0.69 ± 0.03 , also is recorded in Table 6.

Of the characters in Group 1, date of tasseling (A) was positively though not significantly correlated with yield, and date of silking (B) and shrinkage of the harvested ears (C) each were negatively and significantly correlated with yield, when the remaining characters of the group were held constant.⁴

⁴ The term "held constant" is used throughout in the sense that the effect of the variation has been eliminated.

It is interesting to speculate as to why date of tasseling gave a positive partial correlation with yield, and date of silking a negative partial correlation. In order to eliminate any confusing effect of the shrinkage of the harvested ears, partial correlations were computed between date of tasseling and yield for constant date of silking and between date of silking and yield for constant date of tasseling. The first of these correlations ($r_{AX.B}$) was 0.11 ± 0.06 and the second ($r_{BX.A}$) was -0.24 ± 0.05 . Holding either one of these dates constant and permitting the other to vary was equivalent to varying the number of days from tasseling to silking. With the date of tasseling constant, the later silking plants were those with a longer period from tasseling to silking, inasmuch as most of the strains were protandrous. According to the negative partial correlation between yield and date of silking, these plants yielded somewhat less. Similarly, with the date of silking constant, the earlier tasseling plants are those with a longer period from tasseling to silking, which likewise, according to the positive partial correlation between date of tasseling and yield, were less productive. Both of the partial correlations therefore indicate that a decrease in the number of days from tasseling to silking was accompanied by a higher yield. Plants which are decidedly protandrous are difficult to self. The tendency, therefore, in selecting in selfed lines of corn is to obtain lines tasseling and silking more or less simultaneously. These correlations indicate that such a tendency is at least not objectionable.

The partial correlations between yield and two of the characters in Group 2 were positive and between yield and two other characters in this group were negative. One of the positive and one of the negative correlations verged on significance. Plant height (E) was positively associated with yield when chlorophyll grade (D), nodes per plant (F), and nodes below ear (G) were constant. The partial correlation of chlorophyll grade with yield was negative when the other members of Group 2 were held constant. As noted, this indicates that the darker chlorophyll grades were associated with larger yields.

Only one of the members of Group 3 was appreciably correlated with yield when the other members of the group remained constant, and it can not be considered significant. This was ears moldy (K), for which a negative partial correlation of -0.15 ± 0.06 was obtained.

Significant positive partial correlations were obtained between yield and two of the characters of Group 5. The partial correlation between ear length and yield for constant diameter and shelling percentage was 0.31 ± 0.05 , whereas that between ear diameter and yield for constant length and shelling percentage was 0.17 ± 0.06 . The high partial correlation between shelling percentage and yield (0.31 ± 0.05) may have been due in part to the poorly filled ears that occurred in many inbred lines.

These correlations indicate that, for the season in which they were grown, those inbred lines tended to be higher yielding which matured rather early, belonged to the darker chlorophyll grades, were relatively tall, produced relatively many ears (few barren plants), and produced large ears which were longer, rather than greater in diameter, than the average. The ears of the more productive lines also had a high percentage of grain.

SUMMARY SHOWING THE SIGNIFICANT POSITIVE AND NEGATIVE RELATIONS AMONG THE DIFFERENT VARIABLES

Symbol	Variable	Characters with which the variable indicated was significantly correlated in the direction stated		Symbol	Variable	Characters with which the variable indicated was significantly correlated in the direction stated	
		Positive	Negative			Positive	Negative
A	Date of tasseling	BCEFGHMOIPRX	JQS	K	Ears moldy	ILS	JNOX
B	Date of silking	ACEFGHIOIPRX	JQS	L	Suckers per 100 plants	IKMNS	P
C	Shrinkage of the harvested ears	ABEFGHIMNO	JQS	M	Plants with two or more ears	ACEFGJLNO	PQRS
E	Plant height	ABCFGMNOX	JQR	N	Ears per plant	CLMX	KQRS
F	Nodes per plant	ABCEGMNOX	JQR	O	Ear length	ABCEFGIMX	LMNO
G	Nodes below ear	ABCEFGIMNOX	JQR	P	Ear diameter	ABJQRSX	IKMNO
H	Percentage of nodes below ear	ABCFG	JQR	Q	Ear-shape index	JPRS	ABCEFGIMNOX
I	Plants smutted	BCKL	OX	R	Kernel rows per ear	ABJQS	EFGMNO
J	Plants erect at harvest	MPQR	ABCEFGKGO	S	Shelling percentage	IKLPQRX	ABCMO
				X	Yield	ABEFGNOPS	IKQ

COEFFICIENTS OF SIMPLE CORRELATION WITHIN F_1 CROSSES

The coefficients of correlation among the characters within the F_1 crosses are recorded in Table 7. Coefficients three or more times their probable errors are printed in boldface type. The correlations in this table were computed from the data on the F_1 crosses grown in 1926 and, like those within inbred lines, are among characters within the same generation. In all, 461 F_1 crosses from the three 1926 yield groups are represented in these correlations.

A larger percentage of the coefficients of correlation in Table 7 are significant than of those within inbred lines. This is due principally to the larger number of observations, which resulted in smaller probable errors, rather than to material differences in the actual size of the coefficients. Of the 171 correlations recorded, 104 are statistically significant in that they are at least three times their probable errors. A summary of the data in Table 7 is given, showing which characters were correlated significantly either positively or negatively and bringing out more clearly the interrelations among the different variables.

The coefficients of correlation of primary interest are those between yield and the other characters. In general, yield was correlated positively with the characters indicating length of season required to reach maturity, plant vigor, and ear size. It was correlated negatively with the characters indicating the relative susceptibility to disease and with ear-shape index. The correlation between yield and shrinkage of the harvested ears was negative though not significant. This unimportant correlation probably was due to the fact that the season of 1926 was ideal for the ripening of the later kinds of corn, so that practically all of the crosses matured fully.

The highest correlation between yield and the other characters in the F_1 crosses was the correlation of 0.42 with ear length. The correlation of yield with ear diameter was 0.25 and that with ear-shape index was -0.27 . This indicates that although both length and diameter, which go to make up size of ear, were correlated positively with yield, length of ear was a more important factor in determining larger yields than diameter of ear.

Other interesting relations are brought out in Table 7. In general, all of the characters indicating maturity or plant vigor (Groups 1 and 2) were correlated positively among themselves. Most of these characters were correlated negatively with percentage of plants erect at harvest and with ear-shape index. Evidently, the tall, vigorous plants were more likely to go down before harvest and to have long, slender ears.

Additional evidence that the down plants were those having long, slender ears is to be had from (1) the significant positive correlations of plants erect at harvest with ear-shape index and ear diameter and (2) the significant negative correlation of plants erect at harvest with ear length. The correlation already mentioned between ear characters and yield indicated that crosses with short, thick ears were less productive than those with long, slender ears. This might account for their being more erect at harvest, for they were supporting a smaller weight of ear.

In order to determine whether there were any striking differences in the coefficients of correlation in the different yield groups, the correlations between the different characters and yield were com-

puted for each group separately. The coefficients of correlation from each of the three experiments and, for comparison, the correlations for the three groups combined, are recorded in Table 8.

TABLE 8.—*Coefficients of correlation between yield and the various other characters within the different yield groups of F_1 crosses grown in 1926*

Character correlated with yield	Coefficient of correlation for the yield group indicated			
	90 white crosses	90 early yellow crosses	281 later yellow crosses	All 461 crosses of the three groups combined
Date of tasseling	0.16±0.07	0.52±0.05	0.08±0.04	0.18±0.04
Date of silking	.11±.07	.37±.06	.10±.04	.16±.04
Shrinkage of the harvested ears	-.17±.07	.10±.07	-.08±.04	-.06±.03
Plant height	.27±.07	.09±.07	.40±.03	.33±.04
Nodes per plant	.16±.07	.26±.07	.42±.03	.25±.04
Nodes below ear	.15±.07	.24±.07	.37±.03	.31±.03
Percentage of nodes below ear	.04±.07	-.06±.07	.05±.04	.03±.03
Plants smutted	-.09±.07	-.26±.07	-.23±.04	-.22±.03
Plants erect at harvest	-.13±.07	.22±.07	-.11±.04	-.05±.03
Ears moldy	-.28±.07	-.55±.05	.01±.04	-.18±.03
Suckers per 100 plants	-.26±.07	.14±.07	-.02±.04	.00±.03
Plants with two or more ears	.01±.07	-.01±.07	-.02±.04	-.01±.03
Ears per plant	.20±.07	.25±.07	.01±.04	.09±.03
Ear length	.42±.06	.61±.05	.37±.03	.42±.03
Ear diameter	.41±.06	.44±.06	.13±.04	.25±.03
Ear-shape index	-.20±.07	-.27±.07	-.28±.04	-.27±.03
Kernel rows per ear	.07±.07	.29±.07	-.15±.04	-.02±.03
Shelling percentage	.50±.06	.49±.05	.20±.04	.31±.03

For the most part the coefficients of correlation between the various characters and yield (Table 8) are fairly consistent in the different yield groups. They vary somewhat in size in the different experiments, but in only a few cases are they significant and positive in one group and significant and negative in another. The correlation between yield and the percentage of plants erect at harvest is positive in the early yellow crosses and negative in the late yellow crosses. It is negative also in the white crosses, but is not significant. No explanation can be offered for this fact.

Practically the same situation is true for the correlation between kernel rows per ear and yield. It is positive in the early yellow crosses and negative in the later crosses; that is, high yield was associated with the higher rowed sorts of the early corn and the fewer rowed sorts of the later corn. A possible explanation for this may be found in the fact that the mean numbers of kernel rows were 13.4 for the early yellow crosses and 16.4 for the late yellow crosses. Both correlations therefore point to a type intermediate between these groups as better suited to the conditions at Ames in 1926. This explanation is supported by several other correlations, which indicate that late maturity and large ear size were of more importance in determining high yield in the two groups of early crosses, where the ear size was relatively small, than in the group of later crosses with their correspondingly larger ears. Date of tasseling, date of silking, ears per plant, ear length, ear diameter, and shelling percentage were correlated more highly with yield in the two groups of early crosses than in the group of late crosses.

Ears moldy also were correlated with yield more highly in the two groups of early crosses than in the group of late crosses.

COEFFICIENTS OF PARTIAL AND OF MULTIPLE CORRELATION WITHIN F_1 CROSSES

Coefficients of partial and of multiple correlation were computed from the data on the F_1 crosses for the same four groups of variables that were used within the inbred lines (p. 694). Chlorophyll grade, however, was omitted, as no data on this were taken in the F_1 crosses. These correlations for the F_1 crosses are recorded in Table 9.

TABLE 9.—Coefficients of partial and of multiple correlation between yield and four groups of the other characters of the F_1 crosses

Group No.	Designation of coefficient	Coefficients of correlation	Group No.	Designation of coefficient	Coefficients of correlation
1	$r_{AX.BC}$	$0.10 \pm .03$	3	$r_{IX.JK}$	$-0.17 \pm .03$
	$r_{BX.AC}$	$.05 \pm .03$		$r_{JX.IK}$	$-.09 \pm .03$
	$r_{CX.AB}$	$-.11 \pm .03$		$r_{KX.IJ}$	$-.14 \pm .03$
2	$R_{X.ABC}$	$.21 \pm .03$	5	$R_{X.IJK}$	$.26 \pm .03$
	$r_{XX.FG}$	$.23 \pm .03$		$r_{OX.PS}$	$.53 \pm .02$
	$r_{FX.EG}$	$.12 \pm .03$		$r_{PX.OS}$	$.29 \pm .03$
	$r_{GX.EF}$	$.06 \pm .03$		$r_{SX.OP}$	$.33 \pm .03$
	$R_{X.EFG}$	$.41 \pm .03$		$R_{X.OP.S}$	$.61 \pm .02$
				$R_{X.ABFGIKOPS}$	$.71 \pm .02$

As was true for the inbred lines, the multiple correlation computed between the group of ear characters (Group 5) and yield was the highest of the multiple correlations. The multiple correlation between yield and the group of characters indicating plant vigor and size (Group 2) also was high. The remaining two groups of characters were significantly correlated with yield, though the correlations were much lower. The coefficient of multiple correlation between 10 of the characters studied and yield (Table 9) was 0.71 ± 0.02 .

The partial correlations in Group 1 were low, although two of them were significant. The partial correlation of yield with date of tasseling (A) was positive and significant but low, and that with date of silking (B) was not significant. In the inbred lines the partial correlation between yield and date of silking was negative and significant. The partial correlation between shrinkage of the harvested ears and yield, for constant dates of shelling and of silking ($r_{CX.AB}$), was significant although somewhat low.

In Group 2, which was composed of characters indicating plant size and vigor, the partial correlation of yield with each variable was positive. That between plant height and yield ($r_{EX.FG}$) was the highest. The partial correlation between nodes below ear and yield ($r_{CX.EF}$) was too small to be considered significant.

The partial correlations between yield and the characters indicating relative susceptibility to disease (Group 3) were negative. Two of them were significant, and one, between plants erect at harvest (J) and yield, probably was not significant.

All three of the partial correlations between yield and the ear characters in Group 5 were positive and significant statistically. Moreover, they were all higher than the partial correlations between yield and any of the other characters studied in the F_1 crosses.

These correlations indicate that for the season of 1926 those crosses were on an average most productive which tasseled and silked rather late, were comparatively tall, had a relatively large number of nodes per plant, were comparatively free from smutted plants and moldy ears, produced relatively many ears (few barren plants), and produced large ears which were longer, rather than greater in diameter, than the average. The ears of the more productive crosses also had a high percentage of grain. This is in agreement with the results obtained within inbred lines, except that early maturity was associated with productivity within the inbred lines.

CORRELATIONS BETWEEN CHARACTERS OF INBRED PARENTS AND THOSE OF THEIR F_1 CROSSES

The coefficients of correlation between inbred parents and F_1 crosses could be computed for three different relations, (1) that between the characters of the F_1 cross and those of each parent separately, (2) that between the characters of the F_1 cross and the mean value of these characters in the two parents, and (3) that between the characters of the inbred parent and the mean value of these characters in all of their crossbred progeny.

Under the first method of computing the coefficients of correlation the F_1 crosses would be paired first with one parent and then with the other. Each cross, therefore, would appear twice in a correlation table. Under the second method each F_1 cross would be paired with the mean value for its two parents and appear in a correlation table only once. It appeared that there should be a definite relation between the coefficients calculated by the first two methods. Investigation showed that, if there is no correlation between the two inbred parents of the F_1 crosses, then $r_2 = r_1\sqrt{2}$, where r_1 is the correlation with each parent and r_2 is the correlation with the mean of the two parents.

In the present material each inbred line was crossed with enough of the other lines within its group to eliminate practically any possibility of correlation between the parents. The coefficients of correlation between the characters of the F_1 crosses and those of each parent separately could be calculated easier than could the correlations with the mean value of these characters in the two parents. Accordingly, the former correlations were calculated first and the latter were computed by multiplying by $\sqrt{2}$.

COEFFICIENTS OF CORRELATION FOR INDIVIDUAL CROSSES WITH THEIR PARENTS

The coefficients of correlation between the different characters in the F_1 crosses and the same characters in the parent lines are shown in Table 10, the correlations with each parent separately and those with the parental means being in parallel columns.

The correlations were positive and significant in every case. The highest correlation was for percentage of erect plants, although high correlations also were obtained for kernel rows per ear, nodes per plant, nodes below ear, and percentage of nodes below ear. The lowest correlation obtained was for yield. The squared coefficients measure the portion of the variance for the different characters in the crosses that is associated with variation in the parent lines. They therefore indicate those characters for which selection in the parent lines will be more and less effective in determining the type of the crosses.

TABLE 10.—Coefficients of correlation between characters in F_1 crosses and the same characters in the parental inbred lines

[1926 comparison of crosses]

Sym- bol	Character	Coefficients of corre- lation		Sym- bol	Character	Coefficients of corre- lation	
		With each parent separately	With the parental means			With each parent separately	With the parental means
A.....	Date of tasseling.....	0.31±0.02	0.43±0.03	K.....	Ears moldy.....	0.22±0.02	0.31±0.03
B.....	Date of silking.....	.24±.02	.34±.03	L.....	Suckers per 100 plants.	.39±.02	.50±.02
C.....	Shrinkage of the harvested ears.	.25±.02	.35±.03	M.....	Plants with two or more ears.	.18±.02	.25±.03
E.....	Plant height.....	.32±.02	.45±.03	N.....	Ears per plant.....	.26±.02	.36±.03
F.....	Nodes per plant.....	.42±.02	.60±.02	O.....	Ear length.....	.30±.02	.43±.03
G.....	Nodes below ear.....	.42±.02	.60±.02	P.....	Ear diameter.....	.35±.02	.49±.03
H.....	Percentage of nodes below ear.	.41±.02	.58±.02	Q.....	Ear-shape index.....	.34±.02	.48±.03
I.....	Plants smutted.....	.17±.02	.24±.03	R.....	Kernel rows per ear.	.47±.02	.67±.02
J.....	Plants erect at har- vest.	.51±.02	.72±.02	S.....	Shelling percentage.	.39±.02	.55±.02
				X.....	Yield.....	.14±.02	.20±.03

TABLE 11.—Coefficients of correlation between yield in F_1 crosses and various characters in the parental inbred lines

[1926 comparison of crosses]

Sym- bol	Characters in the inbred parent with which yields of the crosses were correlated	Coefficients of corre- lation		Sym- bol	Characters in the inbred parent with which yields of the crosses were correlated	Coefficients of corre- lation	
		With each parent separately	With the parental means			With each parent separately	With the parental means
A.....	Date of tasseling.....	0.12±0.02	0.17±0.03	K.....	Ears moldy.....	−0.07±0.02	−0.10±0.03
B.....	Date of silking.....	.10±.02	.13±.03	L.....	Suckers per 100 plants.	.03±.02	.04±.03
C.....	Shrinkage of the harvested ears.	.05±.02	.07±.03	M.....	Plants with two or more ears.	.07±.02	.10±.03
E.....	Plant height.....	.13±.02	.19±.03	N.....	Ears per plant.....	.08±.02	.12±.03
F.....	Nodes per plant.....	.17±.02	.24±.03	O.....	Ear length.....	.11±.02	.16±.03
G.....	Nodes below ear.....	.14±.02	.20±.03	P.....	Ear diameter.....	.09±.02	.13±.03
H.....	Percentage of nodes below ear.	−.05±.02	−.08±.03	Q.....	Ear-shape index.....	−.10±.02	−.14±.03
I.....	Plants smutted.....	−.06±.02	−.09±.03	R.....	Kernel rows per ear.	.00±.02	.01±.03
J.....	Plants erect at har- vest.	−.04±.02	−.06±.03	S.....	Shelling percentage.	.07±.02	.10±.03
				X.....	Yield.....	.14±.02	.20±.03

Table 11 shows the coefficients of correlation between yields in the F_1 crosses and various characters in the inbred parents.

The correlations in Table 11 are much lower than those in Table 10, as is to be expected. Those characters in the inbred parents which were correlated most highly with yield in the F_1 crosses, in the order of their size, were nodes per plant, yield, nodes below ear, plant height, date of tasseling, and length of ear. These characters are approximate measures of vigor and size in the inbred plants. As the correlations were positive, the larger, more vigorous inbreds produced the higher yielding crosses.

COEFFICIENTS OF CORRELATION FOR INDIVIDUAL PARENTS WITH MEANS OF THEIR CROSSES

Coefficients of correlation between the characters of each inbred parent and the mean yield of all of its crossbred progeny are shown in Table 12. Correlations between the characters in the inbred parents and the mean values of the same character in the crossbred

progeny are shown in Table 13. In computing the coefficients in Table 12, the mean yield of all crosses of each inbred line first was determined. These means then were adjusted for heterogeneity by the method already described (p. 690). The coefficients in Table 13 are reported separately for the five different yield groups. No adjustments were made for heterogeneity within a group.

The coefficients of correlation between characters in the parental inbred lines and the mean yield of the crossbred progeny (Table 12) are shown for the crosses compared in 1926 after the parents had been selfed for three generations, for those compared in 1927 after the parents had been selfed for four generations, and for both groups combined. The correlations for the crosses made after four generations of selfing are no more significant than those for the crosses made after three generations of selfing, although several of the coefficients differed markedly in the two seasons.

TABLE 12.—*Coefficients of correlation between the various characters in the inbred parents and the mean yield of their crossbred progeny*

Character in parent correlated with mean yield of crossbred progeny	Coefficients of correlation for the groups indicated		
	Crosses compared in 1926	Crosses compared in 1927	Both groups combined
Date of tasseling	0.23±0.08	-0.04±0.09	0.15±0.06
Date of silking	.15±.08	-.08±.09	.07±.06
Shrinkage of the harvested ears	.24±.08	-.05±.09	.16±.06
Chlorophyll grade	-.07±.08	-.11±.09	-.08±.06
Plant height	.16±.08	.32±.08	.21±.06
Nodes per plant	.29±.07	.28±.08	.28±.05
Nodes below ear	.24±.08	.22±.08	.22±.06
Percentage of nodes below ear	-.04±.08	.03±.09	-.01±.06
Plants smutted	-.14±.08	-.03±.09	-.11±.06
Plants erect at harvest	-.04±.08	.11±.09	.01±.06
Ears moldy	-.13±.08	.05±.09	-.08±.06
Stuckers per 100 plants	.02±.08	-.13±.09	-.02±.06
Plants with two or more ears	.20±.08	.12±.09	.17±.06
Ears per plant	.20±.08	.08±.09	.16±.06
Ear length	.16±.08	-.23±.08	.02±.06
Ear diameter	.23±.08	-.13±.09	.10±.06
Ear-shape index	-.09±.08	.07±.09	-.04±.06
Kernel rows per ear	.13±.08	-.04±.09	.07±.06
Shelling percentage	.19±.08	-.11±.09	.08±.06
Coefficient of variability of number of kernel rows	.16±.08	.00±.09	.10±.06
Yield	.32±.07	.12±.09	.23±.06

In 1926 there was a late fall. This was advantageous to the crosses requiring a longer season. In 1927, however, there was an early frost, which was disadvantageous to the later maturing crosses. All of the discrepancies between coefficients for the two seasons occurred in those characters which indicate large-sized ears and late maturity, such as date of tasseling, date of silking, shrinkage of the harvested ears, ear length, ear diameter, kernel rows per ear, and shelling percentage. Most of these characters were positively correlated with mean yield of crosses in 1926 but correlated either negatively or not significantly in 1927.

A few of the characters, such as plant height, nodes per plant, nodes below ear, plants with two or more ears, and yield of the inbred parent, were significantly correlated with mean yield of crosses in both groups, separately and combined. Yield of the inbred lines showed the highest positive correlation with mean yield

of crosses in 1926, a positive correlation in 1927, and the second highest positive correlation of both years combined. The highest positive correlation for both groups combined was that for nodes per plant with yield.

The coefficients of correlation between characters in the parental inbred lines and the mean value of the same character in their crossbred progeny (Table 13) are the highest correlations that were obtained in this study. In fact, many of them are high enough to be very valuable for predictive purposes. This, of course, is augmented by the fact that the means of the crosses were used, thereby smoothing out much of the random variation. The fact that the data were not adjusted for heterogeneity within the different yield groups may account for part of the size of these correlations. Varietal differences, however, can not account for the high correlations in the white crosses grown in 1926. In this group, 16 inbred lines were represented in the correlation studies. Three of these lines were from Silver King corn and the remaining 13 were from Four-County White. These two varieties are very closely related, Four-County White being practically a selected strain of Silver King. As the correlations for this group are higher, in general, than those for the other groups, heterogeneity seems to have been a factor of little importance.

TABLE 13.—Coefficients of correlation between characters in the inbred parents and the means of the same characters for all of their crossbred progeny, as computed for each of the five different yield groups

Character	Coefficients of correlation in the yield group indicated				
	White crosses, 1926	Early yellow crosses, 1926	Later yellow crosses, 1926	White crosses, 1927	Yellow crosses, 1927
Date of tasseling.....	0.86±0.04	0.71±0.08	0.65±0.06	0.28±0.19	0.61±0.06
Date of silking.....	.80±.06	.52±.11	.59±.07	.45±.16	.56±.06
Shrinkage of the harvested ears.....	.78±.06	.71±.08	.62±.07	.36±.18	.50±.07
Plant height.....	.52±.12	.63±.09	.53±.08	.59±.13	.60±.06
Nodes per plant.....	.89±.03	.86±.04	.64±.07	-----	-----
Nodes below ear.....	.81±.06	.75±.07	.72±.05	-----	-----
Percentage of nodes below ear.....	.58±.11	.89±.03	.75±.05	-----	-----
Plants smutted.....	.80±.06	.24±.15	.69±.06	-----	-----
Plants erect at harvest.....	.77±.07	.79±.06	.88±.03	.59±.13	.41±.08
Ears moldy.....	.46±.13	.66±.09	.25±.11	.65±.12	.24±.09
Suckers per 100 plants.....	.69±.09	.88±.04	.78±.04	-----	-----
Plants with two or more ears.....	.77±.07	.32±.14	.59±.07	-----	-----
Ears per plant.....	.40±.14	.59±.10	.58±.07	-----	-----
Ear length.....	.37±.14	.67±.09	.80±.04	-----	-----
Ear diameter.....	.98±.01	.78±.06	.71±.06	-----	-----
Ear-shape index.....	.47±.13	.19±.15	.85±.03	-----	-----
Kernel rows per ear.....	.85±.05	.92±.02	.88±.03	-----	-----
Shelling percentage.....	.82±.05	.50±.12	.69±.06	.82±.07	.14±.09
Yield.....	.67±.09	.64±.09	.25±.11	.41±.17	.45±.07

Most of the coefficients in Table 13 are significant. They are all positive and of sufficient size to indicate that the characters of the inbred lines were very definitely expressed in their crossbred progeny. The high correlations in Table 13 emphasize the advantages to be gained by using the mean of several crosses as a criterion of the value of an inbred line.

COEFFICIENTS OF PARTIAL AND OF MULTIPLE CORRELATION BETWEEN CHARACTERS OF INBRED PARENTS AND MEAN YIELDS OF THEIR CROSSBRED PROGENY

Coefficients of partial and of multiple correlation, similar to those computed within the inbred lines and within the crosses, were calculated for four groups of characters of the inbred parents with the mean yields of their crossbred progeny. The groups were the same as in the correlations within inbred lines, except that yield of the inbred parent was substituted for chlorophyll grade in Group 2 (p. 690). The partial and multiple correlations computed are recorded in Table 14.

TABLE 14.—Coefficients of partial and of multiple correlation for four groups of characters of the inbred parents and the mean yields of their crossbred progeny

Group No.	Designation of coefficient ^a	Coefficient of correlation	Group No.	Designation of coefficient ^a	Coefficient of correlation
1	$r_{AY'.BC}$	0.20 ± 0.06	3	$r_{IY'.JK}$	-0.14 ± 0.06
	$r_{BY'.AC}$	$-.13 \pm .06$		$r_{JY'.IK}$	$-.05 \pm .06$
	$r_{CY'.AB}$	$-.20 \pm .06$		$r_{KY'.IJ}$	$-.14 \pm .06$
2	$R_{Y'.ABC}$	$.31 \pm .05$	5	$R_{Y'.IJK}$	$.20 \pm .06$
	$r_{RY'.FGX}$	$-.07 \pm .06$		$r_{OY'.PS}$	$.10 \pm .06$
	$r_{FY'.RGX}$	$.22 \pm .06$		$r_{PY'.OR}$	$.16 \pm .06$
	$r_{OY'.RFX}$	$.03 \pm .06$		$r_{SY'.OP}$	$.12 \pm .06$
	$r_{XY'.RFG}$	$.31 \pm .05$		$R_{Y'.OPS}$	$.28 \pm .06$
	$R_{Y'.RFGX}$	$.42 \pm .05$			

^a Y' symbolizes the mean yields of the crossbred progeny.

The highest of the multiple correlations (0.42) was for mean yield of the crossbred progeny with the members of Group 2, the characters of the inbred parent indicating plant vigor and size. The second highest multiple correlation was between yield and the characters in Group 1. The multiple correlation between yield and the characters in Group 5, which was the highest both within the inbred lines and within the F_1 crosses, was not so important here.

Few of the coefficients of partial correlation between mean yield of crosses and the characters in the different groups with the remaining characters in the group held constant can be considered significant. Two of the coefficients in Group 1, $r_{AY'.BC}$ and $r_{CY'.AB}$, were positive and significant and one, $r_{BY'.AC}$, was negative and not significant. The positive partial correlation for date of tasseling with mean yield of crosses and the negative partial correlation for date of silking with mean yield of crosses, the other characters remaining constant, are in agreement with the partial correlations within the inbred lines and indicate that a negative relation exists also between number of days from tasseling to silking in the inbred parents and the mean yield of their crosses.

Two significant partial correlations were obtained in Group 2. One of these was between number of nodes per plant (F) and mean yield of crosses (Y) and the other was between yield of the inbred line (X) and the mean yield of crosses, each with the other characters in the group constant. The partial correlation for yield of the inbred line with mean yield of crosses for constant plant height, nodes per plant, and nodes below ear indicates that, when size of plant remains constant, approximately 10 per cent of the variation in the mean yield of crosses is associated with variation in yield of the parent line.

None of the partial correlations for mean yield of crosses and the characters in Group 3 and 5 were significant. Inasmuch as all of those for Group 3 were negative they probably indicate that susceptibility to disease in the parent lines tended to be associated with the lower mean yields of crosses. Inasmuch as all of the coefficients of partial correlation for Group 5 are positive, they probably indicate that large-sized ears in the inbred lines tended to be associated with the higher mean yields of crosses.

PREPOTENCY AMONG THE INBRED LINES

The coefficients of correlation between parents and progeny measure the general tendency of these classes toward resemblance in the characters studied. These correlations, however, fail entirely to show the important variation in prepotency that occurs among the inbred lines. As here used, prepotency denotes the capacity of a parent line to impress upon its F_1 crossed progeny certain characteristics, regardless of whether these characteristics are expressed in the parent or not. Variation in prepotency can be shown only by detailed data on the parents and their crossbred progeny. Such data are given in Tables 15 to 26. These are selected for presentation as characteristic of the experiments as a whole.

The experiment for the comparison of inbred lines in 1926 was conducted on more productive soil than that for the comparison of crosses in 1926. The season of 1927 was so much less favorable for corn production than that of 1926 that the acre yields of many of the crosses grown in 1927 were actually less than the yields of some of the better inbred lines grown in 1926. For these reasons, the data on the inbred parent lines, though comparable inter se are not comparable with the data on the crosses.

PRODUCTIVENESS

The data on the yields of the F_1 crosses in the 1926 and 1927 comparisons of crosses and the yields of the inbred parent lines are shown for the various yield groups in Tables 15 to 19. Because of differences in season and soil productivity these yields are not comparable from table to table.

The crossing-block numbers of the parent lines are shown along the top and left sides of the tables. The yield of each cross is recorded at the intersection of the appropriate row and column. The mean yields of the crosses of each parent line and the yields of the parent lines themselves are recorded in rows and columns along the right and lower margins of the tables.

The correlations between the yields of the inbred parents and the mean yields of their F_1 crosses, as given in Table 13, for the five yield groups in Tables 15 to 19, inclusive, were: 0.67 ± 0.09 ; 0.64 ± 0.09 ; 0.25 ± 0.11 ; 0.41 ± 0.17 ; and 0.45 ± 0.07 . The correlations for Tables 15, 16, and 19 are significant, and those for Tables 17 and 18 are not significant. These correlations indicate a relation between the yields of the parents and the mean yields of their crosses. They do not bring out, however, the way in which certain lines impress upon their crosses a consistency in productiveness.

TABLE 15.—Yield per row (pounds) of the F_1 crosses between inbred lines from varieties of white corn compared with yields of the parent lines

[1926 comparison of crosses. P. E. (probable error) of the difference between the yields of any two parent lines, ± 0.460 ; P. E. of the difference between the yields of any two F_1 crosses, ± 0.627 ; P. E. of the difference between means of 9 crosses, ± 0.192 , and between means of 10 crosses, ± 0.182]

No. of parent line	Crossing-block Nos. of parent lines										Mean yield of crosses	Yield of parent ^a
	11	12	13	14	15	16	17	18	19	20		
1	10.87	13.46	9.90	11.75	13.54	11.67	10.85	12.16	11.93	14.61	12.07	-----
2	12.31	12.82	13.94	16.67	13.99	12.68	12.44	12.37	13.72	13.42	13.44	6.88
3	9.18	11.79	9.86	11.79	12.90	9.28	11.71	12.21	10.91	12.26	11.19	4.16
4	12.14	13.23	12.88	15.41	14.17	12.06	13.02	11.84	10.92	14.55	13.02	7.24
5	10.04	13.42	11.61	16.13	13.77	10.48	12.79	12.02	11.38	13.64	12.53	7.37
6	10.55	12.00	11.99	14.14	14.61	12.34	13.11	13.40	11.96	13.71	12.78	4.82
7	12.43	13.82	11.36	14.21	13.63	13.87	14.13	12.28	13.10	13.54	13.24	7.87
9	13.10	12.84	12.74	16.02	14.09	13.82	14.09	13.10	14.97	13.26	13.80	7.53
10	10.67	12.50	13.33	15.59	10.94	12.10	12.08	9.71	9.88	11.59	11.84	5.13
Mean yield of crosses	11.25	12.88	11.96	14.53	13.52	12.03	12.69	12.12	12.09	13.40	12.66	-----
Yield of parent ^a	5.71	4.77	-----	7.89	5.85	5.40	5.59	6.42	5.42	-----	-----	-----

^a Yields of the parent lines are not comparable with the yields of the crosses.

^b Mean yield of all crosses in this group.

TABLE 16.—Yield per row (pounds) of the F_1 crosses between inbred lines from early varieties of yellow corn compared with yields of the parent lines

[1926 comparison of crosses. P. E. (probable error) of the difference between the yields of any two parent lines, ± 0.460 ; P. E. of the difference between the yields of any two F_1 crosses, ± 0.501 ; P. E. of the difference between means of 9 crosses, ± 0.154 , and between means of 10 crosses, ± 0.146]

No. of parent line	Crossing-block Nos. of parent lines										Mean yield of crosses	Yield of parent ^a
	31	32	33	34	35	36	37	38	39	40		
21	6.50	6.50	10.24	8.21	12.27	7.81	10.78	10.06	8.61	10.02	9.10	2.45
22	11.45	9.33	11.67	10.74	12.46	11.49	11.88	13.54	12.00	10.40	11.50	8.19
24	13.28	11.72	12.01	12.44	13.46	12.58	11.29	13.34	13.23	10.72	12.41	4.45
25	15.12	12.63	14.22	14.43	14.86	13.80	13.73	14.34	14.50	12.88	14.05	8.74
26	13.74	12.47	14.90	14.84	14.38	13.51	14.00	13.52	13.70	13.17	13.82	7.92
27	9.61	6.40	10.92	10.40	9.41	10.77	10.60	10.69	10.14	8.00	9.69	4.33
28	13.34	10.83	12.75	12.23	12.72	14.30	11.40	12.09	13.77	10.57	12.40	5.02
29	13.69	8.97	15.04	13.33	12.84	12.33	14.60	14.87	10.31	10.16	12.61	2.58
30	14.01	10.15	14.64	13.65	12.50	15.02	14.08	12.28	12.60	9.92	12.88	5.33
Mean yield of crosses	12.30	9.89	12.93	12.25	12.77	12.40	12.49	12.75	12.10	10.65	12.05	-----
Yield of parent ^a	3.20	1.85	6.63	5.68	9.14	5.85	6.63	7.45	3.09	2.83	-----	-----

^a Yields of the parent lines are not comparable with the yields of the crosses.

^b Mean yield of all crosses in this group.

Each of these tables contains excellent demonstrations of the differences in the ability of different inbred lines consistently to produce high-yielding crosses. In Table 15, inbred lines Nos. 11 to 20 were parents of comparable series of crosses. The crosses of line 14 had the highest mean yield. All crosses yielding 16 pounds or more had line 14 as one parent. A comparison of lines 13 and 14 as parents shows that in every pair of comparable crosses, the one having line 14 as parent yielded more. A similar condition holds when lines 11, 16, and 17 are compared with line 14.

Of the crosses in Table 16, those of line 25 had the highest mean yield. Comparing the individual crosses of line 25 with comparable crosses of lines 21, 22, 24, and 27, the crosses of line 25 were always the highest yielding.

Of the crosses in Table 17 those of line 66 had the highest mean yield. Comparing the crosses of this line with comparable crosses of lines 46, 47, 52, 54, 55, 56, 61, 62, 67, 70, 74, 78, and 80, that with line 66 was the highest yielding in every case.

Now, if it be assumed that the lines listed across the top of Table 17 (41, 42, 43, etc.) were the lines that were being tested, and that the lines listed down the left side of the table were the testers, most of the former lines were used in 28 or 29 comparable crosses. The crosses of line 65 had the highest mean yield. Comparing lines 65 and 63 as parents, there were 28 pairs of comparable crosses, in 27 of which that of line 65 was higher yielding. The yields of these 28 pairs of comparable crosses are shown graphically in Figure 1. Lines 43 and 63 also were used in 28 pairs of comparable crosses, and in 27 of the comparisons the cross of line 43 was the higher yielding.

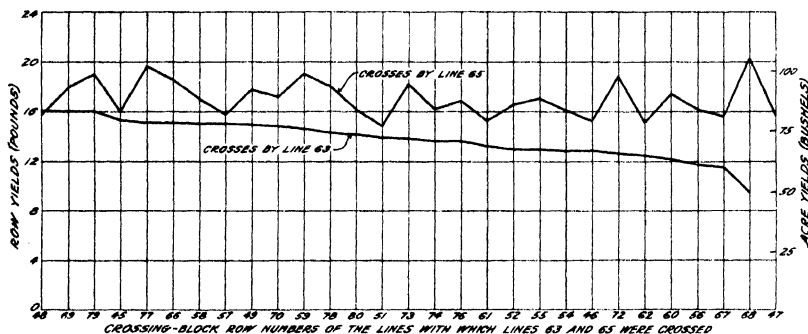


FIGURE 1.—Yields of the comparable F_1 crosses of several inbred lines with line 63 and with line 65

TABLE 17.—Yield per row (pounds) of the F_1 crosses between inbred lines from the later varieties of yellow corn compared with yields of the parent lines

[1926 comparison of crosses. P. E. (probable error) of the difference between the yields of any two parent lines, ± 0.460 ; P. E. of the difference between the yields of any two F_1 crosses, ± 0.627 ; P. E. of the difference between means of 10 crosses, ± 0.182 , and between means of 29 crosses, ± 0.107]

No. of parent line	Crossing-block Nos. of parent lines										Mean yield of crosses	Yield of parent ^a
	41	42	43	50	53	63	64	65	71	75		
45	16.38	15.76	16.81	21.42		15.33	12.79	15.87	18.59	19.39	16.93	8.50
46	13.31	13.64	15.62	13.77	14.31	12.78	11.77	15.16	11.60	19.28	14.12	7.56
47	13.99	13.37	14.60	13.46	12.66		16.14	15.63	15.79	16.78	14.71	5.27
48	14.52	17.60	18.45	10.09	20.74	16.05	15.69	15.73	15.05	15.91	15.98	9.39
49	16.23	19.35	19.02	13.70	16.19	14.86	17.80	17.74	17.08	17.38	16.94	8.91
51	16.61	10.97	18.05	10.94	15.68	13.88	15.71	14.82	16.97	16.89	15.05	7.40
52	13.62	15.14	16.47	14.41	15.92	12.86	13.89	16.49	14.82	15.10	14.87	6.87
54	14.15	16.10	16.67	14.68	14.35	12.83	16.11	15.97	15.98	17.43	15.43	7.64
55	13.81	14.98	15.99	13.80	13.95	12.85	16.33	16.99	16.80	16.68	15.22	5.85
56	13.75	13.19	15.53			11.69		16.09	15.14	15.23	14.37	6.65
57	15.06	17.41	18.01	15.67		15.01	17.68	15.70	17.38	17.59	16.61	8.27
58	14.71	16.49	17.04	16.63	15.00	15.04	18.02	17.04	17.91	15.00	16.29	7.22
59	15.44	18.29	19.93	16.30		14.60	17.29	19.03	19.15		17.50	6.97
60	15.21	16.52	16.43	14.61	17.50	12.07	18.40	17.37	17.61	18.12	16.38	9.84
61	14.80	12.96	16.73	14.67	14.27	13.24	16.75	15.20	14.80	15.05	14.85	5.14
62	13.26	15.85	14.30	14.09	15.23	12.39	16.58	15.11	15.72	14.81	14.73	3.92
66	15.99	17.16	18.30	17.97	19.57	15.07	18.53	18.48	17.19	19.82	17.81	7.36
67	11.83	13.65	15.28	12.44	15.22	11.51	17.82	15.60	13.71	14.13	14.12	11.67
68	11.97	13.11	17.23	16.20	16.48	9.53	18.56	20.54	15.38	15.33	15.41	4.44
69	13.96	16.18	18.29	16.54	18.71	16.04	16.33	17.90	14.51	15.65	16.41	10.26
70	13.64	15.49	18.03	16.02	16.59	14.83	15.98	17.14	15.83	11.72	15.53	8.42
72	12.35	15.47	15.71	15.98	17.28	12.64	16.73	18.76	14.54	16.07	15.55	7.77
73	12.18	18.47	15.43	15.00		13.77	16.23	18.15	11.55	13.54	14.92	6.71
74	14.15	16.12	16.90	17.46	17.45	13.61	15.92	16.14	12.61	13.72	15.41	6.09
76	14.39	15.34	16.89	15.71	15.38	13.57	15.68	16.79	18.65	16.39	15.88	6.34
77	17.14	20.44	16.50	18.03	17.54	15.11	17.83	19.58	13.56	18.34	17.41	9.07
78	13.60	13.36	15.81	15.88	16.39	14.25	17.32	17.98	15.40	17.12	15.71	6.66
79	12.92	12.95	15.81	17.05	18.62	15.99	17.49	18.99	14.96	15.06	15.98	
80	14.70	16.46	17.67	14.73	17.48	14.11	16.93	16.10	16.93	15.71	16.08	8.60
Mean yield of crosses	14.26	15.58	16.81	15.26	16.36	13.77	16.51	16.96	15.70	16.19	15.73	
Yield of parent ^a			7.62	1.83	6.84	8.44	9.62	7.08	8.62	8.66		

^a Yield of the parent lines are not comparable with the yields of the crosses.

^b Mean yield of all crosses in this group.

Of the crosses in Table 18 those of lines 112 and 107 had the highest mean yields. Comparing the crosses by these two lines with comparable crosses by the other lines, the crosses of line 112 outyielded all of the comparable crosses of line 103, and line 111 outyielded eight of the nine crosses by line 104 and outyielded seven of the eight crosses by line 101 and by line 109. The crosses by line 107 outyielded all comparable crosses by line 104 and outyielded the comparable crosses by line 101, by line 103, and by line 106 in eight out of nine cases.

Among the crosses by 43 parent lines listed at the left of Table 19 the crosses by lines 124, 135, and 146 had the highest mean yields. The crosses by line 135 outyielded all of the comparable crosses by each of 20 of the remaining 42 lines. The crosses by line 124 outyielded all of the comparable crosses by 19 of the remaining 42 lines, except that in two pairs the comparable crosses were tied. The crosses by line 146 outyielded all of the comparable crosses by 17 of the remaining 42 lines.

CHARACTERS OTHER THAN PRODUCTIVENESS

Inspection of the data on all of the characters studied showed differences in prepotency similar to those pointed out for productiveness. These differences in prepotency for various characters occurred more or less equally within all of the different yield groups. This generality of prepotency has been shown in detail sufficiently by the data on productiveness in the different groups. Accordingly, additional data are presented here only on selected characters in the group of late crosses compared in 1926.

Data on the percentage of plants erect at harvest are recorded in Table 20.

TABLE 18.—Yield per row (pounds) of the F_1 crosses between inbred lines from varieties of white corn compared with yields of the parent lines

[1927 comparison of crosses. P. E. (probable error) of the difference between the yields of any two parent lines, ± 0.460 ; P. E. of the difference between the yields of any two F_1 crosses, ± 0.563 ; P. E. of the difference between means of 9 crosses, ± 0.173 , and between means of 10 crosses, ± 0.164]

No. of inbred line	Crossing-block Nos. of parent lines											Mean yield of crosses	Yield of parent ^a
	131	102	103	104	105	106	107	109	110	111	112		
101	-----	11.33	11.45	10.60	10.64	10.78	11.33	11.03	11.17	10.97	11.50	11.08	7.17
102	11.33	-----	11.64	12.39	9.54	11.62	12.89	9.25	12.30	13.06	14.54	11.86	5.32
103	11.45	11.64	-----	10.36	11.33	11.67	11.13	11.76	11.40	10.08	11.18	11.20	7.64
104	10.60	12.39	10.36	-----	10.69	10.86	11.87	10.99	11.64	11.31	-----	11.19	7.40
105	10.64	9.54	11.33	10.69	-----	10.50	12.45	10.89	11.91	-----	13.16	11.23	5.11
106	10.78	11.62	11.67	10.86	10.50	-----	13.65	11.35	12.18	10.46	11.88	11.51	6.65
107	11.33	12.89	11.13	11.87	12.45	13.65	-----	12.06	12.87	12.21	12.40	12.29	6.30
109	11.03	9.25	11.76	10.99	10.89	11.35	12.06	-----	12.31	10.87	12.69	11.32	8.18
110	11.17	12.30	11.40	11.64	11.91	12.18	12.87	12.31	-----	12.50	12.57	12.09	6.31
111	10.97	13.06	10.08	11.31	-----	10.46	12.21	10.87	12.50	-----	11.22	11.42	4.26
112	11.50	14.54	11.18	-----	13.16	11.88	12.40	12.69	12.57	11.22	-----	12.35	-----
Mean	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	^b 11.60	-----

^a Yields of the parent lines are not comparable with the yields of the crosses.

^b Mean yield of all crosses in this group.

TABLE 19.—Yield per row (pounds) of the F_1 crosses between inbred lines from varieties of yellow corn compared with yields of the parent lines

[1927 comparison of crosses. P. E. (probable error) of the difference between the yields of any two parent lines, ± 0.460 ; P. E. of the difference between the yields of any two F_1 crosses, ± 0.617 ; P. E. of the difference between means of 9 crosses, ± 0.190 , and between means of 43 crosses, ± 0.086]

No. of parent line	Crossing-block Nos. of parent lines									Mean yield of crosses	Yield of parent ^a
	121	140	143	150	153	157	160	168	171		
114	9.11	11.85	9.27	10.97	9.99	8.76	10.57	10.07	9.99	10.06	4.91
116	10.21	10.04	10.34	10.54	8.82	10.10	10.64	8.67	9.22	9.84	5.24
117	8.76	11.26	10.34	10.79	11.54	11.38	9.93	9.48	10.19	10.41	7.34
118	8.54	12.87	10.70	9.59	11.16	9.44	10.18	10.70	9.65	10.31	5.66
119	9.94	11.67	11.21	9.28	10.70	10.52	10.90	11.18	10.69	10.68	9.41
120	10.17	13.06	9.96	11.08	11.13	11.00	11.59	10.24	11.53	11.08	5.71
123	9.51	10.30	10.18	10.66	11.09	9.14	10.12	10.97	11.22	10.35	9.83
124	10.99	12.75	13.43	10.83	12.46	12.69	12.85	12.68	12.02	12.30	9.38
125	11.73	-----	11.07	10.89	11.18	9.78	11.77	11.01	9.79	10.90	12.17
126	11.68	13.37	13.18	9.50	12.47	10.62	13.21	11.38	11.26	11.85	15.76
128	11.74	11.86	11.62	11.31	12.56	12.27	12.27	11.13	10.69	11.72	8.48
129	10.16	11.94	10.79	11.68	11.52	11.49	10.44	10.92	11.48	11.16	8.87
130	10.32	11.31	10.37	10.14	10.95	10.83	11.08	11.57	11.63	10.91	2.02
132	9.83	12.44	10.61	10.85	11.39	11.10	11.20	10.71	12.05	11.13	12.04
133	9.00	11.23	8.82	8.57	8.96	10.26	9.72	11.73	10.36	9.85	6.89
135	13.21	12.57	12.34	12.06	12.40	14.04	12.99	10.99	11.29	12.43	9.52
136	12.43	10.41	11.54	11.16	13.20	11.41	12.14	12.92	9.67	11.65	7.99
139	12.18	9.81	11.53	11.76	11.43	12.25	12.10	10.29	11.18	11.39	11.21
141	11.80	11.99	12.79	9.31	11.45	10.54	11.55	12.07	9.02	11.17	8.72
142	11.83	11.64	11.38	12.43	11.99	11.63	11.72	12.21	11.06	11.76	6.82
144	12.07	10.32	10.51	11.31	11.20	11.53	11.26	10.98	9.66	10.98	9.43
146	11.54	13.87	13.26	12.49	11.84	10.34	13.72	10.86	12.50	12.27	4.72
147	9.85	10.38	8.13	10.16	10.82	7.90	10.72	10.30	9.88	9.79	4.83
149	10.68	12.02	9.77	9.02	9.09	10.87	10.56	11.01	10.46	10.39	9.53
151	-----	-----	9.72	10.90	10.60	9.02	11.18	9.65	10.05	10.16	7.92
154	10.36	13.05	11.06	11.81	11.31	10.83	11.23	12.54	10.34	11.39	8.42
155	12.67	12.78	9.55	10.58	11.59	11.46	10.44	11.25	10.38	11.19	7.69
156	9.76	10.12	8.98	10.83	10.44	12.29	11.05	10.00	10.91	10.49	6.88
158	9.73	11.97	10.95	12.00	9.12	8.74	10.71	10.16	10.10	10.39	6.28
159	9.44	9.37	8.96	8.27	8.93	8.62	6.57	8.58	9.11	8.65	5.13
161	10.09	12.81	11.16	10.83	12.23	9.86	11.24	9.89	11.66	11.09	8.51
162	10.76	9.74	8.49	10.10	9.26	10.50	8.50	8.93	10.39	9.63	8.38
164	9.17	13.19	9.13	11.26	10.83	9.54	10.64	9.30	9.48	10.30	3.71
165	9.25	11.69	9.53	9.68	9.62	9.36	9.52	9.24	9.83	9.75	6.21
166	10.68	10.26	11.55	10.63	10.69	9.50	10.88	8.90	9.61	10.30	10.74
167	10.22	12.48	10.41	9.82	10.62	9.49	9.80	9.24	10.47	10.28	2.14
169	10.88	12.70	10.94	9.64	10.65	11.16	11.21	9.50	11.57	10.92	9.48
170	8.97	9.22	9.42	9.54	7.31	10.01	10.46	7.12	9.05	9.01	4.83
172	9.93	8.90	10.20	11.06	10.05	10.95	10.87	11.15	9.35	10.27	5.20
173	9.00	8.63	9.53	9.66	8.96	8.65	10.78	8.81	7.58	9.07	2.80
174	9.80	11.64	11.67	11.33	11.45	10.10	10.63	10.61	10.03	10.81	-----
175	9.54	-----	9.70	10.28	11.06	8.88	10.36	8.96	10.36	9.89	-----
176	8.51	10.98	9.96	9.97	9.86	9.49	9.87	9.81	10.51	9.88	-----
Mean yield of crosses.	10.38	11.46	10.56	10.57	10.79	10.43	10.92	10.41	10.40	^b 10.65	-----
Yield of parent ^a -----	8.48	9.38	11.58	8.98	5.28	6.51	9.15	5.56	5.21	-----	-----

^a Yields of the parent lines are not comparable with the yields of the crosses.

^b Mean yield of all crosses in this group.

The coefficient of correlation for the percentage of plants erect at harvest, between the parent lines and the means of their crossbred progeny, from the data in Table 20, was 0.88 ± 0.03 (Table 13). This is a very high correlation and indicates that about 77 per cent of the variance among the mean percentages of erect plants in the crosses was associated with variation in percentages of erect plants in the parent lines. All of the crosses of some inbred lines had high percentages of erect plants, whereas all of the crosses of other lines had low percentages of erect plants. Thus, all of the crosses by line 46 had higher percentages of erect plants than the comparable crosses by line 47. A similar condition exists as between lines 57 and 58 and lines 62 and 60. Lines 63 and 65 were used in 28 comparable pairs of crosses. In 27 of these the cross with line 65 as one parent

had a higher percentage of erect plants than that with line 63 as a parent. The data on the percentage of erect plants in these two lines are shown graphically in Figure 2.

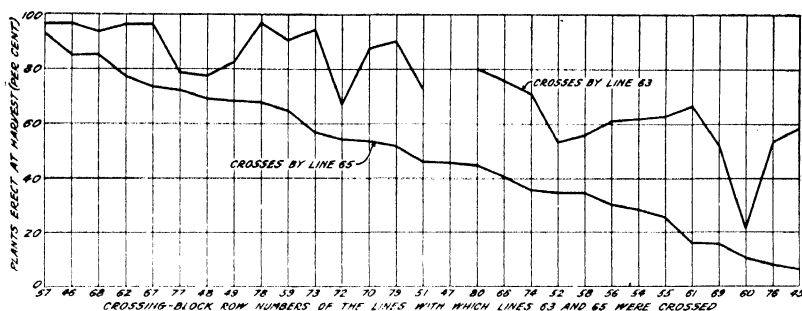


FIGURE 2.—Percentages of plants erect at harvest in the comparable crosses of several inbred lines with line 63 and with line 65

TABLE 20.—Percentage of plants erect at harvest in the F_1 crosses between inbred lines from the later varieties of yellow corn compared with the parent lines

[1926 comparison of grosses]

No. of parent line	Crossing-block Nos. of parent lines										Mean of crosses	Percentage erect in parent ^a
	41	42	43	50	53	63	64	65	71	75		
45	89.6	63.4	32.9	77.9	-----	58.0	50.0	6.3	64.6	72.2	57.2	49.1
46	96.6	92.3	82.5	92.0	70.5	96.7	95.1	85.2	68.9	96.4	87.6	92.1
47	28.9	13.8	42.5	80.9	42.2	-----	19.6	45.3	45.8	72.6	43.5	9.9
48	77.3	50.0	71.8	70.4	100	77.4	60.9	69.2	76.5	72.2	72.6	61.2
49	99.6	97.5	75.2	100	100	82.7	80.8	68.3	83.8	97.8	88.6	55.0
51	67.9	53.5	74.6	81.6	97.7	72.6	56.8	45.9	64.7	80.5	69.6	63.2
52	80.2	57.4	49.0	50.4	81.5	53.1	44.9	34.7	35.5	69.5	55.6	15.2
54	58.7	46.3	15.5	66.5	44.4	61.3	36.6	28.3	56.7	69.9	48.4	8.5
55	77.0	66.4	39.2	61.6	74.5	62.5	5.4	25.7	50.6	79.2	54.2	12.5
56	78.4	58.3	66.0	-----	-----	60.9	-----	30.1	67.8	73.8	62.2	33.9
57	92.0	83.8	94.5	96.6	-----	96.9	80.5	93.0	94.7	98.5	92.3	93.6
58	75.0	42.5	23.3	54.0	68.6	55.6	30.0	34.6	33.8	69.0	48.6	14.4
59	83.7	77.7	74.8	97.7	-----	90.6	63.8	64.6	65.5	-----	77.3	90.2
60	76.6	41.1	30.3	68.8	48.9	21.1	43.7	10.2	41.2	73.7	45.6	52.3
61	66.1	44.3	36.8	41.7	51.1	66.2	49.4	16.0	42.4	67.6	48.2	0.0
62	94.0	95.3	86.1	98.0	99.2	96.4	90.9	77.4	97.3	97.2	93.2	91.3
66	64.8	56.2	65.0	56.3	93.8	75.9	75.6	40.3	78.0	73.5	67.9	1.6
67	95.5	85.3	61.4	83.8	100	96.2	72.1	73.8	73.4	85.4	82.7	80.9
68	93.5	78.0	67.3	97.2	98.1	93.2	86.2	84.8	98.6	77.0	87.4	100
69	84.3	55.4	54.2	56.2	84.0	51.5	52.6	15.7	56.1	65.4	57.5	14.2
70	94.0	89.1	46.5	89.1	83.0	87.9	84.8	53.4	93.7	86.9	80.8	78.2
72	92.1	89.5	62.7	73.5	96.4	67.3	65.0	54.0	62.8	86.7	75.0	65.7
73	99.1	73.8	84.2	100	-----	94.4	83.9	56.5	89.8	96.0	86.4	100
74	76.1	86.7	52.0	80.9	91.7	70.9	38.4	35.4	62.9	89.5	68.4	36.2
76	53.9	49.0	25.3	70.5	79.2	53.5	56.2	8.0	40.6	65.2	50.1	8.3
77	95.0	81.4	62.4	82.8	92.8	78.3	69.5	72.3	76.0	92.8	80.3	88.4
78	95.4	85.4	84.9	97.6	98.5	97.0	74.1	67.8	81.0	96.8	87.8	99.1
79	90.3	84.0	64.8	95.5	72.2	90.0	51.8	51.6	81.3	82.6	76.4	-----
80	84.4	82.0	58.9	85.4	85.7	80.2	54.5	44.6	78.6	86.0	74.0	44.3
Mean of crosses	81.4	68.3	58.1	78.8	81.4	74.6	59.8	48.0	67.7	81.2	^b 69.7	-----
Percentage erect in parent ^a	-----	-----	27.4	78.7	94.8	61.8	37.7	1.5	60.5	85.3	-----	-----

^a The data on the parent lines are not comparable with those on the crosses.

^b Mean percentage erect for all crosses in this group.

Data on the date of silking, recorded as the number of days after June 30, are shown in Table 21. The coefficient of correlation for the date of silking between the parent lines and the means of the crossbred progeny was 0.59 ± 0.07 . These data again illustrate the degree to which inbred lines may influence their F_1 crosses. For example, in the 28 pairs of comparable crosses by lines 71 and 63 the crosses by line 71 silked later in 27 pairs, and in 1 pair the comparable crosses silked on the same day.

TABLE 21.—Date of silking (days after June 30) for the F_1 crosses between inbred lines from the later varieties of yellow corn compared with parent lines

[1926 comparison of crosses]

No. of parent line	Crossing-block Nos. of parent lines										Mean of crosses	Date of silking for parent ^a
	41	42	43	50	53	63	64	65	71	75		
45	23.5	24.5	24.5	26.0	---	23.5	24.0	24.5	28.0	23.5	24.7	32.3
46	25.0	23.5	24.5	27.0	26.0	23.5	22.5	25.0	30.0	24.5	25.2	34.3
47	26.0	24.5	25.5	26.5	26.0	---	24.5	25.5	26.5	25.5	25.6	30.3
48	23.0	25.0	24.0	25.0	24.0	25.0	23.5	23.0	29.0	25.0	24.6	35.3
49	25.0	23.5	23.0	26.0	25.5	24.0	22.5	24.0	27.5	25.0	24.6	34.7
51	25.0	24.0	25.5	28.0	24.5	24.5	26.0	27.5	30.0	28.0	26.3	34.3
52	26.0	26.5	28.0	28.0	27.0	26.0	27.0	28.5	27.0	26.0	27.0	38.3
54	25.0	23.5	25.5	22.5	24.0	24.0	26.0	26.0	25.5	26.0	24.8	31.0
55	23.0	25.0	24.5	26.5	24.5	24.0	26.0	27.0	25.0	25.0	25.0	31.3
56	24.5	23.0	24.5	---	---	23.5	---	25.0	27.0	25.0	24.6	29.3
57	25.0	24.0	25.5	23.0	---	23.5	26.5	24.0	25.5	27.0	24.9	31.7
58	24.0	26.0	27.0	26.5	25.0	24.5	27.5	28.0	27.0	27.5	26.3	30.3
59	26.0	25.0	28.0	25.5	---	24.5	26.0	27.0	27.0	---	26.1	30.0
60	26.0	26.0	30.5	29.0	29.0	24.0	30.5	32.0	28.5	29.0	28.4	32.0
61	27.0	29.0	28.0	27.0	28.0	25.5	29.5	30.5	28.0	27.0	28.0	36.3
62	23.5	22.5	28.0	23.5	23.5	23.5	24.5	27.5	26.5	26.0	24.9	30.7
66	27.0	25.0	30.5	28.0	26.0	24.5	27.5	28.5	28.0	29.5	27.4	38.3
67	29.0	24.5	30.5	27.0	27.0	27.5	29.0	29.5	31.0	27.0	28.2	33.0
68	26.0	24.5	30.0	24.0	26.0	25.0	26.0	29.0	28.0	29.0	26.8	34.3
69	28.0	26.0	31.0	29.0	28.5	26.0	27.5	30.0	29.0	29.0	28.4	35.7
70	23.5	23.5	26.0	27.0	24.0	23.0	24.5	26.0	26.0	29.0	25.2	34.3
72	27.5	26.5	29.5	27.0	27.5	29.0	29.0	28.0	30.0	30.5	28.4	35.0
73	30.5	28.0	30.5	29.0	---	27.0	31.0	30.5	33.5	33.0	30.3	43.0
74	30.0	27.0	33.0	30.0	29.5	28.0	28.5	34.0	34.5	33.0	30.8	38.3
76	29.5	26.5	30.0	30.0	27.0	25.0	28.0	30.5	30.5	30.5	28.8	39.0
77	30.0	26.0	33.0	29.0	28.0	27.5	28.0	31.0	27.5	29.0	28.9	38.3
78	27.0	24.0	29.0	27.5	25.0	23.5	23.5	26.5	26.5	28.0	26.0	36.7
79	29.0	28.5	31.0	28.5	30.0	27.0	29.0	30.5	33.0	33.0	30.0	---
80	24.5	23.0	28.0	25.0	26.0	24.5	23.5	27.5	26.0	27.0	25.5	30.7
Mean of crosses	26.2	25.1	27.9	26.8	26.3	25.0	26.5	27.8	28.3	27.8	^b 26.8	---
Date of silking for parent ^a	---	---	36.3	33.0	35.0	30.3	32.3	38.0	40.0	39.0	---	---

^a The data on the parent lines are not comparable with those on the crosses.

^b Mean date of silking for all crosses in this group.

Data on the number of days between tasseling and silking are recorded in Table 22. Protandry is indicated by positive values and protogyny by negative values. The coefficient of correlation between inbred parents and the mean value for their crossbred progeny for the data in Table 22 was 0.66 ± 0.06 . (This coefficient is not shown in Table 13.)

TABLE 22.—Days from tasseling to silking in the F_1 crosses between inbred lines from the later varieties of yellow corn compared with the parent lines

[1926 comparison of crosses]

No. of parent line	Crossing-block Nos. of parent lines.										Mean of crosses	Value for parent ^a
	41	42	43	50	53	63	64	65	71	75		
45	-1.0	2.5	1.0	1.0		0.5	1.0	1.0	1.5	1.5	1.0	-0.4
46	.5	1.5	1.0	.5	1.5	0	0	2.0	2.0	1.0	1.0	-.2
47	2.0	2.5	1.0	2.0	3.0		2.0	2.5	2.0	2.0	2.1	1.6
48	-2.0	2.0	.5	1.0	1.0	1.5	.5	0	.5	2.0	.7	2.0
49	-.5	1.5	0	-1.0	.5	.5	1.0	1.0	.5	.5	.4	3.7
51	.5	2.0	1.0	2.5	1.0	1.5	2.5	3.0	1.0	2.0	1.7	3.3
52	0	3.5	2.5	1.0	1.5	2.0	1.5	2.5	1.5	1.0	1.7	1.6
54	.5	1.5	1.5	.5	1.0	1.0	1.0	3.0	.5	2.5	1.3	.3
55	.5	3.5	.5	2.0	1.5	1.0	3.0	3.0	1.0	1.5	1.8	-.7
56	.5	1.0	.5			.5		1.0	3.0	1.0	1.1	-.7
57	0	1.0	.5	0		.5	1.0	1.0	-1.0	0	.3	.4
58	-1.0	2.5	2.0	-.5	1.5	1.5	3.0	3.0	2.5	2.5	1.7	.3
59	1.0	2.0	3.0	1.5		2.5	1.5	2.5	1.5		1.9	2.0
60	1.0	3.0	1.5	2.5	2.0	1.0	3.5	3.0	2.0	3.0	2.2	3.0
61	1.0	3.5	.5	1.5	.5	0	3.5	3.0	-.5	1.0	1.4	3.6
62	.5	2.0	1.0	.5	1.5	1.5	2.5	2.5	3.0	2.5	1.8	1.7
66	2.5	2.5	2.0	1.0	1.5	1.5	2.0	2.0	2.0	2.5	2.0	3.3
67	1.0	2.5	.5	1.5	2.0	2.5	5.5	3.5	5.0	2.5	2.6	3.0
68	3.0	3.0	3.5	1.5	3.0	2.0	3.0	5.5	5.5	5.0	3.5	5.6
69	2.5	.5	2.0	-.5	1.5	1.0	1.5	2.5	1.0	2.0	1.4	1.0
70	.5	3.0	0	3.0	1.0	1.5	2.5	3.0	2.5	4.5	2.2	3.3
72	3.5	5.5	4.0	4.0	3.5	4.5	6.0	4.0	6.0	5.5	4.6	5.7
73	0	4.0	5.0	1.5		3.5	3.5	1.5	2.5	3.0	2.7	6.7
74	1.5	3.5	4.0	1.0	3.0	2.0	4.0	3.5	4.0	4.0	3.0	3.3
76	1.5	4.5	1.5	1.0	3.0	2.0	4.0	4.5	4.5	4.0	3.0	4.3
77	1.0	1.5	2.0	-.5	1.5	1.0	2.0	1.5	1.0	2.5	1.4	2.0
78	0	0	2.0	0	1.0	1.5	.5	1.5	1.5	2.5	1.0	.7
79	2.5	5.0	4.0	3.0	3.0	3.5	4.5	3.5	5.0	4.0	3.8	
80	1.5	2.5	3.0	2.0	2.5	2.0	2.0	3.0	2.5	3.5	2.4	4.0
Mean of crosses	.8	2.5	1.8	1.2	1.8	1.6	2.4	2.5	2.2	2.5	2.0	
Value for parent ^a			3.0	-2.0	2.0	.6	.6	4.3	3.7	3.0		

^a The data on the parent lines are not comparable with those on the crosses.^b Mean number of days from tasseling to silking for all crosses in this group.

Here, too, different inbred lines transmitted very definite tendencies to their F_1 crosses. The mean number of days from tasseling to silking for the crosses by line 41 was 0.8. For those by line 42 the mean was 2.5. In 25 of the 29 pairs of comparable crosses of which these two inbred lines were parents, the cross by line 42 required more days from tasseling to silking than did the comparable cross by line 41; in three pairs the crosses were tied; and in only one pair did the cross by line 41 require more days from tasseling to silking than the cross by line 42. An interesting example of a possible effect of the selection within selfed lines is the number of lines and crosses which are protogynous. Of the progenies listed in Table 22, 5 of the inbred lines and 10 of the F_1 crosses silked before they tasseled. The general condition among the parent varieties grown in the locality where these data were obtained is for the plants to begin shedding pollen from one to three days before silks appear. It is possible that in selecting the inbred lines there has been an unconscious tendency toward relatively earlier silking plants, as these are the most convenient for self-pollinating. This may have been a factor in obtaining the protogynous lines.

TABLE 23.—Plant height (in feet) of the F_1 crosses between inbred lines from the later varieties of yellow corn compared with the parent lines

[1926 comparison of crosses]

No. of parent line	Crossing-block Nos. of parent lines										Mean of crosses	Plant height of parent ^a
	41	42	43	50	53	63	64	65	71	75		
45	7.25	8.00	8.25	8.00	-----	7.50	8.00	8.50	9.00	8.25	8.08	5.00
46	7.25	9.00	8.25	8.25	8.50	8.00	8.75	9.00	9.25	8.25	8.45	7.67
47	7.00	8.00	8.25	7.75	8.00	-----	8.00	9.00	9.00	8.75	8.19	6.50
48	6.00	7.50	8.00	8.00	7.50	6.75	7.75	8.50	8.00	7.50	7.55	7.17
49	7.50	8.00	8.25	8.00	8.00	7.75	8.25	8.50	9.00	8.25	8.15	7.00
51	7.75	8.25	8.50	8.25	8.00	8.00	9.00	9.00	9.25	8.75	8.48	7.17
52	7.00	8.50	8.50	7.75	8.25	7.75	8.50	9.50	8.50	8.25	8.25	6.67
54	6.25	8.00	8.00	7.25	7.00	7.00	8.00	8.50	8.00	8.25	7.62	5.67
55	6.25	8.00	7.75	7.00	7.75	7.00	8.00	8.50	8.25	8.00	7.65	6.17
56	6.50	7.25	7.50	-----	-----	7.00	-----	8.00	8.50	7.75	7.50	6.33
57	6.50	8.00	7.50	7.50	-----	7.75	7.75	7.50	8.25	8.00	7.64	8.50
58	7.00	8.50	7.75	8.00	8.00	7.50	8.50	9.00	9.00	8.50	8.18	6.17
59	7.00	7.75	8.00	7.75	-----	7.00	7.75	9.00	9.00	-----	7.91	6.50
60	7.00	8.25	8.25	7.50	8.00	7.00	8.75	8.50	8.50	8.25	8.00	6.83
61	7.25	8.00	8.00	7.25	8.25	7.00	8.25	8.00	8.75	8.25	7.90	6.00
62	6.75	7.75	7.75	7.50	7.75	7.00	7.50	7.75	8.50	8.00	7.62	6.00
66	7.50	8.25	8.75	8.25	8.00	7.25	8.25	8.50	8.50	8.00	8.12	6.33
67	6.25	8.25	7.50	8.00	8.00	8.75	8.50	8.50	8.50	7.50	8.00	7.00
68	5.75	8.00	7.75	8.25	7.75	7.25	8.25	8.50	8.50	7.50	7.75	5.67
69	6.50	8.00	7.75	8.25	8.00	7.75	8.25	8.50	8.50	8.50	8.00	7.33
70	6.25	8.25	8.00	8.25	8.00	7.00	8.25	8.25	8.00	7.75	7.80	6.67
72	6.50	8.75	8.75	8.50	8.00	7.50	9.00	9.00	7.75	8.25	8.20	7.43
73	7.50	8.50	8.00	8.25	-----	7.75	9.25	9.25	9.00	8.50	8.44	7.33
74	7.00	8.25	8.00	8.75	8.50	7.50	8.50	8.75	8.50	7.75	8.15	7.17
76	7.00	8.00	7.50	8.25	8.25	7.25	8.25	8.25	8.50	8.00	7.92	7.67
77	7.50	9.00	8.50	8.75	8.75	7.50	9.50	9.75	9.00	8.25	8.65	8.33
78	6.50	8.25	7.75	8.25	8.00	7.50	8.50	9.25	9.00	8.00	8.10	7.00
79	7.75	8.50	8.00	8.75	9.50	8.00	9.00	9.25	9.50	8.50	8.68	-----
80	6.75	8.25	7.75	8.00	8.00	7.75	8.00	8.25	8.50	7.50	7.90	7.17
Mean of crosses	6.86	8.17	8.02	8.01	8.07	7.46	8.37	8.65	8.64	8.11	^b 8.03	-----
Plant height of parent ^a	-----	-----	7.00	7.00	7.00	5.33	7.83	7.67	7.33	7.33	-----	-----

^a The data on the parent lines are not comparable with those on the crosses.^b Mean plant height for all crosses in this group.

The data on plant height are given in Table 23. The coefficient of correlation between parents and the means of their crosses was 0.53 ± 0.08 . Of the various lines shown, line 41 was outstanding in that all of its F_1 crosses were short. It is possible that this line was homozygous for dominant genes determining short stature. The lines other than 41 differed but little in the mean heights of their F_1 crosses. Even so, there were consistent tendencies in the crosses by certain lines. As an example, the mean height of crosses for line 64 was 0.91 foot greater than that of line 63. These two lines were used in 27 pairs of comparable crosses, and in 25 pairs the cross by line 64 was the taller.

The data on percentage of ears moldy are recorded in Table 24. The parent progeny correlation for this character was only 0.25 ± 0.11 and can not be considered significant. The detailed data, however, show that the crosses by different inbred lines exhibited wide differences and very definite tendencies in regard to the percentage of moldy ears in the harvested crop. Nearly all of the crosses by some lines had a high percentage of moldy ears, whereas in others the per-

centage was low. In the 24 pairs of comparable crosses by line 53 and by line 75, all of the crosses by line 53 had lower percentages of moldy ears. In the 24 pairs of comparable crosses by line 53 and by line 43, 23 of the crosses by line 53 had the lower percentages of moldy ears.

TABLE 24.—Percentage of ears moldy in the F_1 crosses between inbred lines from the later varieties of yellow corn compared with the parent lines

[1926 comparison of crosses]

No. of parent line	Crossing-block Nos. of parent lines										Mean of crosses	Percentage of moldy ears in parent ^a
	41	42	43	50	53	63	64	65	71	75		
45.....	15.7	7.7	23.9	8.0	3.2	15.1	0.0	32.3	12.3	5.9	13.4	26.4
46.....	7.0	14.8	8.2	5.9	3.2	14.0	8.3	6.8	4.7	16.9	9.0	5.5
47.....	0	14.5	22.4	4.0	6.7	8.6	7.2	4.0	13.8	9.0	24.3	40.5
48.....	17.3	6.0	21.4	12.5	4.5	25.6	21.9	9.2	12.9	36.0	16.7	22.8
49.....	4.8	6.9	9.6	5.3	7.8	15.5	9.1	8.3	6.2	17.8	9.1	36.8
51.....	6.0	8.1	11.3	8.3	3.0	12.4	13.0	17.9	13.1	12.0	10.5	21.3
52.....	11.5	7.2	17.7	7.1	8.9	18.1	7.9	10.5	9.9	21.1	11.2	22.6
54.....	7.6	6.6	31.0	7.5	1.9	13.1	10.3	11.1	8.4	14.5	17.4	30.4
55.....	26.5	9.9	27.0	7.4	19.4	24.1	6.8	19.8	12.3	21.1	8.6	26.4
56.....	3.7	15.3	11.2	8.5	7.0	9.0	8.4	9.9	4.2	6.7	9.3	7.3
57.....	5.0	6.8	8.6	2.8	7.0	11.5	4.2	18.6	3.7	12.4	7.1	73.4
58.....	4.5	7.7	17.5	7.0	11.5	6.4	9.8	5.8	4.2	11.2	7.0	31.9
59.....	7.9	3.5	4.8	11.1	12.2	11.5	3.0	6.6	4.5	10.5	9.2	30.1
60.....	5.7	6.8	11.1	3.0	9.0	19.0	7.6	10.4	7.6	16.0	8.1	26.9
61.....	3.1	10.3	8.7	6.3	2.4	12.3	3.3	7.4	7.1	4.5	7.1	26.4
62.....	7.1	8.3	12.1	8.4	5.4	12.2	17.9	15.8	14.7	38.4	15.6	26.7
66.....	18.7	14.0	12.7	16.4	7.7	15.2	5.8	14.1	11.3	22.6	13.3	12.7
67.....	9.2	7.9	22.4	4.6	4.8	6.8	2.7	4.3	6.1	9.8	7.6	13.1
68.....	8.0	9.0	19.6	14.5	13.1	24.1	11.8	17.8	14.3	26.7	16.6	38.5
69.....	6.7	14.9	22.5	13.3	2.7	20.8	18.6	23.8	11.1	29.5	17.6	23.9
70.....	19.2	9.5	27.7	9.8	3.8	16.0	9.1	8.0	8.6	18.7	11.5	17.5
72.....	6.5	9.4	24.9	9.6	5.9	4.0	3.5	4.2	12.4	7.6	19.1	14.6
73.....	3.1	10.2	15.1	8.2	14.2	11.4	8.5	9.7	35.4	13.8	34.8	26.2
74.....	11.7	9.6	24.4	5.0	16.7	8.2	7.8	3.5	14.4	11.0	8.7	3.1
76.....	7.5	12.8	26.9	15.5	8.6	22.9	9.8	12.6	10.9	25.7	17.7	26.2
77.....	12.6	16.2	42.2	6.7	7.4	5.7	11.2	8.3	8.7	11.8	9.2	25.6
78.....	4.8	5.4	16.8	4.7	0	6.7	10.1	5.5	7.9	23.4	11.4	25.6
79.....	10.1	10.1	13.0	8.6	5.6	14.9	16.2	10.2	9.7	20.4	11.4	25.6
80.....	9.7	8.0	10.8	8.2	6.3	14.3	9.2	11.3	8.3	18.2	11.3	25.6
Mean of crosses.....	9.0	9.6	18.1	8.2	6.3	14.3	9.2	11.3	8.3	18.2	11.3	25.6
Percentage of moldy ears for parent ^a			9.0	9.0	7.7	25.6	23.2	18.4	14.4	64.5		

^a The data on the parent lines are not comparable with those on the crosses.

^b Mean percentage of ears moldy for all crosses in this group.

The data in this table afford good illustrations of how certain inbred lines may transmit uniformly to their offspring, characters that they do not themselves express. For example, line 58 had the highest percentage of moldy ears (73.4), and yet the mean percentage of moldy ears for the crosses by this line was lower than the mean for comparable crosses by any other line except those by line 59. Moreover, all of the individual crosses by line 58 were low in percentage of ears moldy, with the exception of that with line 43.

TABLE 25.—*Ear-shape index (diameter÷length) for the F₁ crosses between inbred lines from the later varieties of yellow corn compared with the parent lines*

[1926 comparison of crosses]

No. of parent line	Crossing-block Nos. of parent lines										Mean of crosses	Ear-shape index of parent ^a
	41	42	43	50	53	63	64	65	71	75		
45	0.207	0.218	0.203	0.204	0.260	0.240	0.163	0.187	0.224	0.233	0.209	0.178
46	0.228	0.232	0.210	0.226	0.261	0.195	0.214	0.238	0.242	0.231	0.270	
47	0.240	0.270	0.222	0.239	0.258	0.201	0.200	0.261	0.238	0.236	0.296	
48	0.227	0.221	0.200	0.230	0.248	0.254	0.196	0.187	0.242	0.234	0.221	
49	0.225	0.246	0.230	0.219	0.244	0.269	0.212	0.198	0.269	0.240	0.235	
51	0.241	0.261	0.239	0.237	0.258	0.269	0.220	0.224	0.277	0.257	0.248	
52	0.243	0.244	0.220	0.223	0.228	0.261	0.212	0.203	0.274	0.253	0.236	
54	0.229	0.238	0.230	0.224	0.252	0.280	0.224	0.200	0.254	0.239	0.237	
55	0.253	0.276	0.252	0.256	0.274	0.312	0.234	0.222	0.282	0.268	0.263	
56	0.242	0.253	0.219			0.295		0.197	0.256	0.266	0.247	
57	0.265	0.267	0.244	0.249		0.259	0.217	0.292	0.274	0.269	0.260	
58	0.250	0.251	0.234	0.252	0.262	0.282	0.215	0.202	0.264	0.264	0.328	
59	0.258	0.243	0.211	0.244		0.269	0.207	0.193	0.251	0.234	0.234	
60	0.236	0.275	0.241	0.249	0.272	0.275	0.217	0.219	0.257	0.246	0.249	
61	0.205	0.220	0.204	0.197	0.221	0.245	0.185	0.187	0.250	0.232	0.215	
62	0.275	0.269	0.270	0.253	0.300	0.326	0.237	0.235	0.296	0.274	0.305	
66	0.239	0.240	0.224	0.246	0.263	0.289	0.225	0.207	0.287	0.289	0.251	
67	0.208	0.219	0.210	0.209	0.230	0.227	0.168	0.178	0.224	0.228	0.210	
68	0.227	0.235	0.204	0.215	0.235	0.260	0.188	0.180	0.244	0.235	0.222	
69	0.253	0.267	0.245	0.243	0.270	0.291	0.225	0.232	0.279	0.260	0.256	
70	0.262	0.266	0.223	0.243	0.263	0.299	0.209	0.208	0.266	0.268	0.251	
72	0.253	0.285	0.262	0.256	0.296	0.308	0.236	0.219	0.290	0.300	0.270	
73	0.268	0.249	0.247	0.258		0.320	0.240	0.239	0.315	0.269	0.367	
74	0.231	0.241	0.217	0.224	0.223	0.240	0.218	0.194	0.279	0.274	0.274	
76	0.283	0.266	0.233	0.255	0.271	0.321	0.219	0.211	0.271	0.251	0.258	
77	0.262	0.267	0.241	0.252	0.267	0.293	0.221	0.209	0.266	0.264	0.254	
78	0.222	0.242	0.218	0.207	0.228	0.256	0.183	0.183	0.239	0.224	0.220	
79	0.264	0.255	0.215	0.236	0.222	0.246	0.197	0.188	0.267	0.247	0.233	
80	0.239	0.239	0.224	0.240	0.230	0.266	0.215	0.210	0.263	0.254	0.238	
Mean of crosses	0.242	0.250	0.227	0.235	0.253	0.275	0.210	0.208	0.264	0.254	0.242	
Ear-shape index of parent			0.237	0.240	0.338	0.360	0.207	0.199	0.305	0.286		

^a The data on the parent lines are not comparable with those on the crosses.^b Mean ear-shape index for all crosses in this group.

The data on ear-shape index are shown in Table 25. The correlation between parent and mean of crossbred progeny was 0.85 ± 0.03 . Ear-shape index was obtained by dividing the mean ear diameter by the mean ear length. A large index indicates an ear whose diameter was large relative to its length, whereas a small one indicates a relatively long slender ear. The index means for the crosses by various inbred lines ranged from 0.275 for line 63 to 0.208 for line 65. The data in Table 1 show that most of this difference was due to the difference in ear length. The mean ear length for the crosses by line 65 was 5 cm. greater than that for the crosses by line 63, whereas the mean ear diameter of the crosses by line 63 was only 0.15 cm. greater than that of the crosses by line 65.

The data on the mean number of kernel rows per ear are recorded in Table 26. The correlation between the parent and the mean of crossbred progeny was 0.88 ± 0.03 . The correlations for this character were uniformly high in all of the three yield groups for which it was computed. (Table 13.) The mean number of kernel rows per ear for the individual crosses listed in Table 26 ranged from 12.1 to 21.7. The means for the crosses by various inbred lines ranged from 14.0 to 18.8. The different inbred lines exercised very definite effects on their crosses. Line 64 produced crosses with relatively low num-

bers of kernel rows per ear, whereas line 63 produced crosses with relatively high numbers of kernel rows per ear. These two inbred lines were used in 27 pairs of comparable crosses, and in every pair the cross by line 63 had a higher number of kernel rows per ear than that by line 64. The numbers of kernel rows for these 27 pairs of comparable crosses and for the parent lines 63 and 64 are shown graphically in Figure 3.

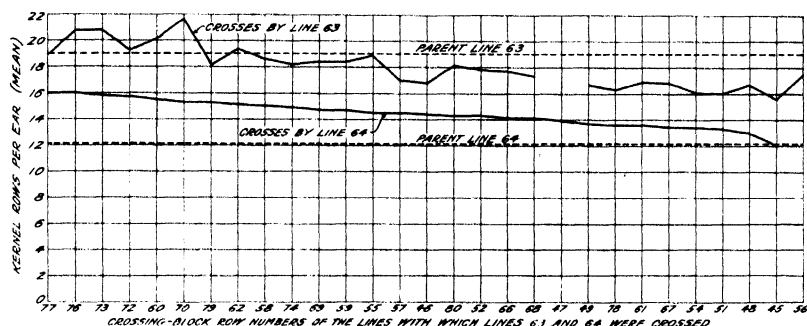


FIGURE 3.—Number of kernel rows per ear in the comparable crosses of several inbred lines with line 63 and with line 64, and the numbers of kernel rows in the parents

TABLE 26.—Mean number of kernel rows per ear for the F_1 crosses between inbred lines from the later varieties of yellow corn compared with the parent lines

[1926 comparison of crosses]

No. of parent line	Crossing-block Nos. of parent lines										Mean of crosses	Kernel rows of parent ^a
	41	42	43	50	53	63	64	65	71	75		
45	15.3	13.4	13.5	13.8		15.6	12.1	13.2	14.5	14.3	14.0	10.6
46	16.1	15.9	16.6	16.5	15.8	16.8	14.4	15.3	16.9	16.2	16.0	16.0
47	16.5	16.0	16.2	15.4	16.5		13.9	15.9	16.9	16.0	15.9	13.9
48	15.7	15.5	14.5	14.6	15.4	16.7	13.0	14.0	15.9	14.0	14.9	12.4
49	15.5	15.1	15.6	14.5	15.2	16.7	13.7	14.8	16.2	15.2	15.2	14.7
51	15.7	14.8	15.6	14.8	14.0	16.0	13.3	14.3	15.7	15.2	14.9	13.8
52	16.8	16.0	16.6	15.8	16.1	17.8	14.3	15.9	17.5	16.9	16.4	14.3
54	15.6	15.1	15.8	15.4	14.7	16.1	13.4	14.3	15.3	14.9	15.1	12.9
55	17.6	16.3	18.1	15.2	16.4	18.9	14.5	16.7	17.6	17.8	16.9	17.5
56	16.1	15.8	16.6			17.5		15.0	17.8	16.6	16.5	15.5
57	16.6	17.4	16.8	15.7		17.0	14.5	19.0	17.5	16.5	16.8	16.4
58	18.3	16.4	17.3	17.9	16.1	18.6	15.0	16.8	17.6	17.5	17.2	16.4
59	19.3	17.9	18.5	16.5		18.4	14.7	16.0	18.0		17.4	16.0
60	17.4	17.4	17.6	17.7	16.9	20.1	15.5	16.4	18.6	16.3	17.4	17.4
61	15.5	15.7	16.1	15.1	13.8	16.9	13.6	14.6	16.8	15.5	15.4	12.7
62	17.9	16.6	17.0	17.0	17.1	19.4	15.1	17.0	18.2	16.7	17.2	16.8
66	16.3	16.5	16.5	15.8	15.6	17.7	14.1	15.2	17.1	15.9	16.1	16.7
67	15.7	16.0	16.6	16.3	14.7	16.8	13.4	15.2	16.1	15.0	15.6	13.7
68	16.0	14.9	16.4	14.9	14.9	17.3	14.1	15.9	16.3	15.1	15.6	14.2
69	16.8	16.1	17.3	16.6	16.4	18.4	14.7	15.1	16.8	16.5	16.5	15.5
70	19.6	18.9	19.3	18.9	18.9	21.7	15.3	17.5	18.7	18.8	18.8	20.4
72	17.9	17.4	18.5	17.9	17.3	19.2	15.7	17.3	19.4	17.7	17.8	21.0
73	19.0	17.2	18.3	18.8		20.8	15.8	18.4	19.5	18.2	18.4	19.3
74	16.7	16.1	17.0	16.9	15.5	18.2	14.9	16.0	17.5	16.4	16.5	14.2
76	18.7	17.9	18.0	17.7	17.3	20.8	16.0	17.1	18.2	18.2	18.1	17.6
77	18.6	17.7	17.6	17.9	16.5	18.9	16.0	16.5	18.0	18.3	17.5	18.3
78	15.1	15.3	15.8	14.7	14.1	16.3	13.6	15.1	16.4	15.2	15.2	12.9
79	17.2	17.1	16.1	17.9	17.2	18.1	15.3	16.9	18.5	18.1	17.3	
80	16.0	15.5	15.2	14.9	15.4	18.1	14.3	14.7	16.7	15.4	15.6	16.4
Mean of crosses	16.9	16.3	16.7	16.2	15.9	18.0	14.4	15.9	17.2	16.4	^b 16.4	
Kernel rows of parent ^a			13.6	15.1	13.0	19.0	12.1	14.6	15.9	15.2		

^a The data on the parent lines are not comparable with those on the crosses.

^b Mean number of kernel rows per ear for all crosses in this experiment.

DISCUSSION

The relations studied may be considered more conveniently in groups comprising (1) correlations among characters within a single generation, (2) those between characters in the inbred parent lines and the same characters in the crossbred progenies, and (3) correlations between the various characters in the inbred parents and the yield of the crossbred progenies. The interpretation of the individual coefficients of correlation was discussed as the data were presented. Emphasis here will be placed upon the more general relations.

The coefficients of correlation among characters within the same generation indicate the extent to which the different characters were associated under the conditions of the experiment and the relative ease of obtaining various combinations of characters. Of the correlations within inbred lines and within F_1 crosses, always within the same generation, those of yield with the other characters are of most interest. The different characters (indicated by their symbols) which were correlated significantly with yield are listed in Table 27.

TABLE 27.—*Different characters (indicated by their symbols) which were correlated significantly with yield*

Inbreds, crosses, and individual parents compared	Characters correlated significantly with yield	
	Positively	Negatively
Within the inbred lines.....	ENOPS.....	BCDQ.
Within the F_1 crosses.....	ABEFGNOPS.....	JKQ.
Individual crosses and parents (1926 comparison of crosses).....	ABEFGNOPX.....	Q.
Individual parents and mean yields of crossbred progenies (1926 and 1927 comparisons of crosses).....	EFGX.....	

In general, characters indicating vigor and size were correlated positively with yield in the inbred lines and in the crosses. The apparent exception, the reversal of relation for date of silking (B) in the two groups, probably is due to the fact that late silking among inbred lines frequently reflects a lack of inherent vigor. In the crosses, on the other hand, date of silking is more largely a measure of relative earliness. The negative relation of yield and shrinkage of the harvested ears (C) in the inbred lines is another expression of this condition. The negative relation between yield and ear-shape index (Q) shows clearly that in these experiments the larger yields were composed of relatively long, slender ears.

The coefficients of correlation between characters in the inbred parent lines and the same characters in the crosses show the extent to which these parental characters were expressed in the crosses. They are of importance, therefore, in determining those characters for which selection in the inbred lines was more and less effective as a means of obtaining them in the crosses. These correlations fail to bring out differences in the prepotency of the different parent lines. Such differences can be observed only from detailed comparisons of the crosses of the individual parent lines.

All of the correlations between the same characters in the parent and in the progeny were positive. Those between characters of the inbred parents and the means for the same characters in the crossbred

progenies (Table 13) were very high, and indicate that, on an average, the characters of the parent were very definitely expressed in the crossbred progeny. Some parent lines impressed upon their crossbred progeny certain characters not expressed in the parents. In general, however, it appears that those inbred lines should be selected as parents whose characters conform most closely to those desired in the cross.

The coefficients of correlation between various characters in the inbred parent lines and the yield of the crossbred progenies indicate the degree to which these characters in the inbred parent influence the yield of the crossbred progeny. The different parental characters (indicated by their symbols) which were correlated significantly with yield of the crossbred progeny are listed in Table 27.

The positive correlations between yield in the crosses and so many of the characters in the inbred line which are indicative of plant vigor are encouraging. Most lines that have been inbred for a number of generations are lacking in vigor and productiveness to a degree which would make the commercial production of F_1 seed expensive. These correlations indicate that the most productive crosses may be expected from the most productive inbred parents. Large yields from the inbred parents will, of course, make for the most economical production of crossed seed.

There may be two more or less distinct objects in comparing inbred lines in different crossbred combinations: (1) To locate high-yielding individual F_1 crosses and (2) to locate inbred lines which give relatively high yields in all combinations. The immediate use intended for the inbred lines will determine the object of any particular comparison. If two inbred lines are wanted for use in an F_1 cross for commercial corn production, then the highest yielding combination may be desired. If the inbred lines are to be used in double crosses, multiple crosses, or in synthetic varieties, however, those lines which produce relatively high yields in practically all crossbred combinations would be the more promising.

Neither of two inbred parent lines which happen to "nick" well need carry many dominant favorable factors. It is only necessary that the few such factors they do carry supplement each other. It would not be expected, however, that such a condition would result in these lines producing high-yielding crosses in many different combinations. Inbred lines which produce high yields in most of the crosses in which they are used either must carry a few uncommon but important dominant favorable factors which supplement those brought in by the general run of inbred lines in the experiment, or they must carry many of the common dominant favorable factors. In either event, the highest yields would be expected to be found among the crosses between lines both of which produced high-yielding crosses in many combinations.

From the data presented on yield (Tables 15 to 19), it is evident that inbred lines differ greatly in their ability to produce high-yielding crosses. Some inbred lines (14, 25, 66, 112, and 135) produced high-yielding crosses in practically all combinations. Other lines (10, 31, 68, 102, and 141) produced some high-yielding crosses and some low-yielding crosses. Still other lines (3, 21, 67, 104, and 159) produced crosses practically all of which were low-yielding. Those lines whose crosses had low mean yields produced few high-yielding individual

combinations. In discarding these lines on the basis of the means of their crossbred progenies, therefore, there would be small chance of eliminating superior germ plasm. What has been said of yield is true also of the other characters studied.

The uniformly high yields of the crosses of some of the inbred lines are very encouraging as an indication of what may be expected from the corn-breeding program under way. The prepotency shown by the different lines in their F_1 crosses suggests that even after only three or four generations of selfing the lines must be homozygous for many of the factors that make for yield and other desirable characters. The higher yielding crosses do not appear to have been random combinations but occurred very definitely among the crosses by certain outstanding parent lines. On the basis of these yield comparisons, it seems possible to predict with reasonable assurance that future crosses by some of the lines tested will outyield comparable crosses by many of the other lines, so that the latter may be discarded without danger of serious loss. Furthermore, the crosses among the better lines retained should be more productive, on an average, than those so far compared, and new combinations among the better parent lines from the different groups may be superior to any so far obtained.

SUMMARY

Coefficients of correlation are reported (1) among characters within the same generation in inbred lines of corn and in F_1 crosses between inbred lines, (2) between characters of the inbred parent lines and the same characters in the crossbred progeny, and (3) between characters of the inbred parent lines and the yield of the crossbred progeny.

Within the inbred lines yield was correlated significantly and positively with plant height, number of ears per plant, ear length, ear diameter, and shelling percentage, and it was correlated significantly and negatively with date of silking, shrinkage of the harvested ears, chlorophyll grade, and ear-shape index.

Within the F_1 crosses yield was correlated significantly and positively with date of tasseling, date of silking, plant height, number of nodes per plant, number of nodes below ear, number of ears per plant, ear length, ear diameter, and shelling percentage, and it was correlated significantly and negatively with percentage of plants smutted, percentage of ears moldy, and ear-shape index.

Positive correlations between characters in the inbred parents and the same characters in the crossbred progeny were obtained for all of the 19 different characters studied. The correlations between characters of the inbred parent and the mean values of the same characters in the crossbred progeny were sufficiently high in many cases to be of value for predictive purposes.

Yield of the F_1 cross was correlated significantly and positively with the following characters in the parents: Date of tasseling, date of silking, plant height, number of nodes per plant, number of nodes below ear, number of ears per plant, ear length, ear diameter, and yield. It was correlated significantly and negatively with ear-shape index in the parents.

The mean yield of the crossbred progeny was correlated significantly and positively with plant height, number of nodes per plant, number of nodes below ear, and yield of the inbred parent line.

Different inbred lines showed marked differences in prepotency for practically all of the characters studied.

The high-yielding crosses do not appear to have been chance combinations but occurred very definitely among the crosses by certain outstanding parent lines.

The extreme productivity of the crosses of some of the inbred lines included in these experiments is promising and indicates what may be expected from this program of corn breeding.

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EXPERIMENTAL STUDIES OF THE ETIOLOGY OF COMMON WARTS IN CATTLE¹

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OCCURRENCE

The common wart, *Verruca vulgaris*, has been described as a specific type of epithelial overgrowth, nonmalignant in character. It is well known that warts are of common occurrence in animals and in man. Adults may be affected, but the young are evidently far more susceptible to these cutaneous growths.

Warts may occur on any part of the body, but in different species they seem to have certain preferred locations. In bovines the primary location of the warts depends somewhat on the age of the animal. In cows the warty growths are usually found on the teats or some other part of the udder, while in calves or yearlings the growths most frequently have their origin on parts of the head, sides of the neck, or shoulders. Occasionally the warts in young cattle may spread from the point of origin to other parts, as the sides, back, or abdomen, eventually involving large areas of the skin surface.

In calves these warty growths may become large and pendulous, sapping the strength and greatly inhibiting the growth of the animal affected. In some cases warts disappear spontaneously without apparent cause or without recourse to treatment of any kind.

ECONOMIC IMPORTANCE

As no figures are available it would be difficult even to approximate the total number of cattle affected annually with warts. It may be stated, however, that available data on the number of warty hides encountered in some of the larger slaughtering establishments indicate that the affection is probably increasing. One of the large slaughtering establishments has estimated that from 15 to 25 per cent of the hides have been found affected at certain periods, particularly during the summer season.

As to the damage caused by warts, tanners state that the final effects of such growths on the hides of cattle are somewhat similar to those observed in "grubby" hides; i. e., holes are found in the hides after tanning, rendering the parts affected practically worthless. The total number of warty hides found annually is unquestionably large, and, since these hides are greatly reduced in value, the yearly loss is sufficiently large to make it of considerable economic importance to the leather industry of the country.

¹ Received for publication Mar. 29, 1929; issued November, 1929.

SUGGESTED CAUSES

As in other types of neoplastic growths, different theories have been advanced relative to the cause of warts. Various forms of external irritation, such as injuries, chemical irritants, etc., have been suggested as causes of warty growths. Following the theory of injury as a possible cause, some have claimed that warts are seen more frequently in branded than in unbranded cattle. Some pathologists, as Ewing (2),² for example, consider warts to be of the nature of inflammatory proliferations.

PREVIOUS EXPERIMENTS

It is commonly believed at present that warts are infectious, and the fact that investigators have been able to transmit them experimentally from animal to animal and from man to man indicates that the belief is probably well founded.

A study of the literature shows that very little experimental work has been done in this country on the transmission of warts in animals. In Europe, however, a number of experiments have been recorded. M'Fadyean and Hobday (4), were successful in reproducing warts in the region of the mouths of dogs by means of experimental inoculations. Royère (5) successfully transmitted warts in different species of animal, including the horse, dog, and calf. Schultz (6) mentions an experiment in which intracutaneous inoculations were made on the hands of three persons with wart material from the udder of a cow, resulting after a long period of incubation in small, warty growths at the points of inoculation.

A number of experiments have been recorded in which warts have been produced, particularly in human beings, by intracutaneous injections with filtrates of wart material. Ciuffo (1) produced warts in a single case in man by inoculation with wart filtrate that had passed through a Berkefeld N candle. Wile and Kingery (7) transmitted warts in a number of cases, in human beings, by intradermic inoculations with sterile filtrates of wart material. Kingery (3), continuing these experiments, was also able to produce warts in the second generation with filtrates. While experimental warts may have been produced in animals also, by inoculations with wart filtrates, the writer has been unable thus far to find any record of such experiments.

PRESENT EXPERIMENTS

Since it had apparently been satisfactorily proved by previous experiments that warts could be transmitted from animal to animal in different species, including the bovine, the experiments here described were undertaken to ascertain with what degree of frequency, or regularity, it would be possible to transmit such growths in cattle, and, more particularly, to obtain information with regard to the nature of the etiological factor involved.

A series of intracutaneous inoculations of cattle with emulsified wart material from different species was undertaken and a like number of inoculations were made with filtrates obtained from the same series of wart specimens. A limited amount of bacteriological work was also included in the experiments.

² Reference is made by number (italic) to "Literature cited," p. 737.

SOURCE OF WART MATERIAL

The wart specimens of bovine origin, used for inoculation purposes, were obtained from cattle-slaughtering establishments at Chicago, Ill., and Fort Worth, Tex. The specimens were received at different times and usually only one or two specimens at once. Although the points of origin of the cattle from which the specimens were obtained are not known, it is probable that these animals came from widely separated sections of the country.

EXPERIMENTAL ANIMALS

On account of their susceptibility it was thought best to use only young cattle, so far as possible, for inoculation purposes. Consequently, with the exception of two adult cows, all the animals used were calves, ranging in age from 5 to 12 months. Most of the calves, however, were from 6 to 8 months of age at the time of inoculation.

The inoculations were made from time to time as fresh wart material became available, and extended over a period of approximately one year. In all, 22 cattle were used in the experiments.

PREPARATION OF WART MATERIAL FOR INOCULATION PURPOSES

Each specimen of warty skin was first thoroughly washed under running tap water for several minutes to remove accumulations of dirt, necrotic material, etc. A number of the warts, large and small, were next clipped from the skin, including a small portion of the latter at the base of the growths. The warts were cut into small bits and thoroughly ground up, with a small amount of sterile, physiologic salt solution, in a sterile mortar. More of the salt solution was then added and the material permitted to stand for a number of hours, or overnight, at refrigerator temperature.

The fluid portion of the emulsified wart material was then poured off. A portion of this constituted the unfiltered material used for inoculation, and the filtrate was obtained in each case by passing the remaining portion of the fluid, or wart extract described above, through a bacteria-retaining filter, Berkefeld N candle, under negative pressure.

Cultural tests of the filtrates were made for sterility, aerobically and anaerobically, with uniform negative results.

TECHNIC EMPLOYED IN ANIMAL INOCULATIONS

Small areas of the skin, at the base of the ear and on the side of the neck near the shoulder were chosen for inoculation. (Fig. 1.) The hair over the area to be inoculated was removed by clipping and shaving, and the skin was washed with soap and water and rinsed with distilled water. Just before the inoculations were made the excess moisture was removed from the shaved surfaces with sterile absorbent cotton.

The inoculation procedure was the same with both the unfiltered materials and filtrates. The skin was scarified over an area from 1 to 2 inches in diameter and small amounts of the wart fluid applied to the surface. At the same time a few drops of the fluid were injected intradermically, at a number of different points, with a fine hypodermic needle.

All the experimental animals were kept isolated during these experiments.



FIGURE 1.—Experimental warts produced on calf 1399 by inoculation: A, Wart at base of ear; B, warts at both sites of inoculation

CULTURAL TECHNIC

The technic followed in culturing the warts was as follows:

The under surface of the skin at the base of the warts was rendered sterile by searing with a hot spatula. Small blocks of the wart tissue were obtained by cutting into the base of the warts with a sterile knife. The pieces of wart tissue were then transferred to both aerobic and anaerobic culture media and incubated at 37.5° C. for several days.

DISCUSSION OF EXPERIMENTAL RESULTS

For the sake of brevity the experimental results have been condensed by arranging the findings for the individual animals from month to month in tabular form. (Tables 1 and 2.) While the tables show only the monthly progress of the experiments, observations of the experimental animals were made at more frequent intervals. The tables show 11 experimental animals in each group.

By referring to Tables 1 and 2 the reader will observe that positive results were obtained in the animals inoculated with both the filtrates and unfiltered wart emulsions from five of the nine original wart specimens used for inoculation purposes. In two cases positive results were obtained only in the calves inoculated with the unfiltered material, and inoculations from specimens CH 2 and FW 3 gave entirely negative results. The two mature cows used in the experiments were inoculated from the FW 3 specimen.

Inoculations made from experimental warts on calf 1403 (fig. 2) gave positive results with both the filtrate and the unfiltered wart material, but when inoculations were made from experimental-filtrate warts on calf 1402, the second transfer of the warts was accomplished only with the filtrate. (Figs. 3 and 4.)

In comparing the inoculation results in the two groups of experimental animals the reader will note that a slightly higher percentage of positive results was obtained with the unfiltered wart material than with the filtrates. Attention may also be called to the fact that in a few cases the experimental warts appeared earlier, and seemed to show more rapid development, in the calves inoculated with the unfiltered material, than in those inoculated with filtrates from the same source. This may be taken to indicate that the filtrates were rendered less active as a result of the filtering process.

However, until we have more definite knowledge regarding the etiological factor involved, it is difficult to explain any apparent variations in the results in the two groups of experimental animals.

The experimental warts which attained a fair stage of development were typical in their clinical appearance, and those examined histologically showed a structure (figs. 5 and 6) characteristic of papillomatous growths.

In regard to the detailed studies made, it is desired to direct especial attention to only two of the experimental calves, Nos. 1402 and 1403. These calves were both inoculated from wart specimen FW 2, 1402 with the wart filtrate, and 1403 with the unfiltered wart material; this was the only instance in which both calves inoculated from the same wart specimen showed well-developed experimental warts, thus giving opportunity for comparative study of the filtrate warts and those produced with the unfiltered wart material. The culturing of the experimental warts in these two calves gave negative bacteriological results in both cases.

TABLE 1.—Results of inoculations of cattle on ear and neck with unfiltered wart material

Specimen	Animal No.	Condition at end of—						
		First month	Second month	Third month	Fourth month	Fifth month	Sixth month	Seventh month
CH 1.....	1333	Skin thickened at point of inoculation on neck.	Some evidence of growth on neck.	Flat growth about 1 inch in diameter on neck.	Growth increased; about 2 inches in diameter.	Growth decreasing in size.	Growth rapidly disappearing.	Little remaining evidence of growth.
CH 2.....	1336	No growth.	No growth.	No growth.	No growth.	No growth.	No growth.	Considered negative.
FW 1.....	1401	Skin thickened at point of inoculation on ear.	Ear growth larger than a pea.	Ear growth slightly increased in size.	Ear growth $\frac{1}{2}$ inch in diameter; no growth on neck.	Ear growth about 1 inch in diameter.	Little change in size of growth; treated with tincture iodine.	Growth practically disappeared.
FW 2.....	1403	No growth.	Small growth size of pea at seat of inoculation on neck.	Shoulder spreading; small growth on ear.	Marked increase in size of growths on ear and neck.	Growth on neck $2\frac{1}{2}$ by 4 inches; ear growths $1\frac{1}{2}$ by 2 inches; calf died.		
CH 3.....	1418	do.	Skin rough at seats of inoculation.	Several small flat growths on neck 1 inch in diameter.	Three growths at base of ear $\frac{1}{2}$ inch in size; growths on neck 2 inches in diameter.	Growth somewhat larger; treated with tincture iodine.	Growths disappeared after treatment.	
CH 4.....	1414	do.	A few small growths on ear and neck.	Ear growths about $\frac{3}{4}$ inch in diameter; slight increase in growth on neck.	Thick growth on ear about 1 inch in diameter; four small growths on neck $\frac{1}{2}$ inch in diameter.	Growth treated with acetic acid.	Growth practically disappeared.	
CH 5.....	1416	Skin thickened at points of inoculation.	Slight evidence of growths on ear and neck.	Several growths on ear and neck little larger than a pea.	Little increase in size of growths on ear and neck.	Growth receding in size.	Growths entirely disappeared.	
FW 3.....	1303	No growth.	No growth.	No growth.	No growth.	No growth.	Considered negative.	
From experimental calf 1403.	1337 (over)	do.	A few minute growths at seats of inoculation.	One wart about $\frac{1}{2}$ inch in diameter on ear; several pea-sized growths on neck.	Growth slightly increased in size on ear and neck.	Growths on both ear and shoulder rapidly disappearing.	Growths entirely disappeared.	
FW 4.....	1339	Skin thickened at points of inoculation.	Some evidence of growths on ear and neck.	A number of well-formed warts on neck and ear.	Flat, spreading growths on neck; one large and several small warts on ear.	Growth on neck about 1 inch; ear growth increasing in size.	Large, well-developed wart on ear 2 by 3 inches in size; growth on neck increased in size.	Warty growths remained about same at time animal was released.
From experimental calf 1402.	1429	No growth.	No growth.	No growth.	No growth.	No growth.	No growth.	Considered negative.

• The animals were calves unless otherwise noted.

TABLE 2.—Results of inoculations of cattle on ear and neck with wart filtrate

Specimen	Animal No. ^a	Condition at end of—						
		First month	Second month	Third month	Fourth month	Fifth month	Sixth month	Seventh month
CH 1	1334	No growth	No growth	No growth	No growth	No growth	No growth	Considered negative.
CH 2	1335	do	do	do	do	do	do	Do.
FW 1	1400	do	Skin thick and rough on neck.	Small, flat growth about 1½ inches in diameter.	Flat, spreading growth on neck 2 inches in diameter.	Growth no larger; no growth on ear.	Growth rapidly receding in size.	Growth has practically disappeared.
FW 2	1402	do	Indications of growth at seat of inoculation on neck.	Flat growth on neck 1 inch in diameter; a few small growths on ear.	Growth on neck increasing in size and thickness.	Well-developed, warty growth on neck 2 by 4 inches; ear growths remained small.	Little change in size of growths; calf slaughtered.	
CH 3	1417	do	No growth	Thickened and rough appearance of skin at seat of inoculation.	A number of small warty growths on neck and at base of ear.	Growths no larger than a pea; appear to be receding in size.	Only two small growths remaining on neck; one on ear.	Growths entirely disappeared.
CH 4	1413	do	A few small growths at seat of inoculation on ear.	Growths on ear disappearing; no growth on neck.	Growths entirely disappeared.			
CH 5	1415	do	No growth	Skin appeared slightly nodular at points of inoculation.	A number of pea-sized growths at base of ear and on neck.	No apparent change in size of growths.	Growths on ear and neck rapidly disappearing.	Do.
FW 3	1289 (cow)	do	do	No growth	No growth	No growth	Considered negative.	
From experimental calf 1403.	1398	do	Some evidence of very small growths on ear and neck.	A number of growths no larger than a pea at both seats of inoculations.	No increase in size of warty growths.	All growths practically disappeared.	No remaining evidence of growths.	
FW 4	1427	do	No growth	No growth	No growth	No growth	No growth	Considered negative.
From experimental calf 1402.	1428	Skin appeared thick over inoculated areas.	Some evidence of growths on ear and neck.	Numerous small growths on neck; a few at base of ear.	Growths on neck larger, covering area of 2 by 3 inches; ear growths remained small.	Calf found dead; slight increase in size of growths on neck.		

^a The animals were calves unless otherwise noted.

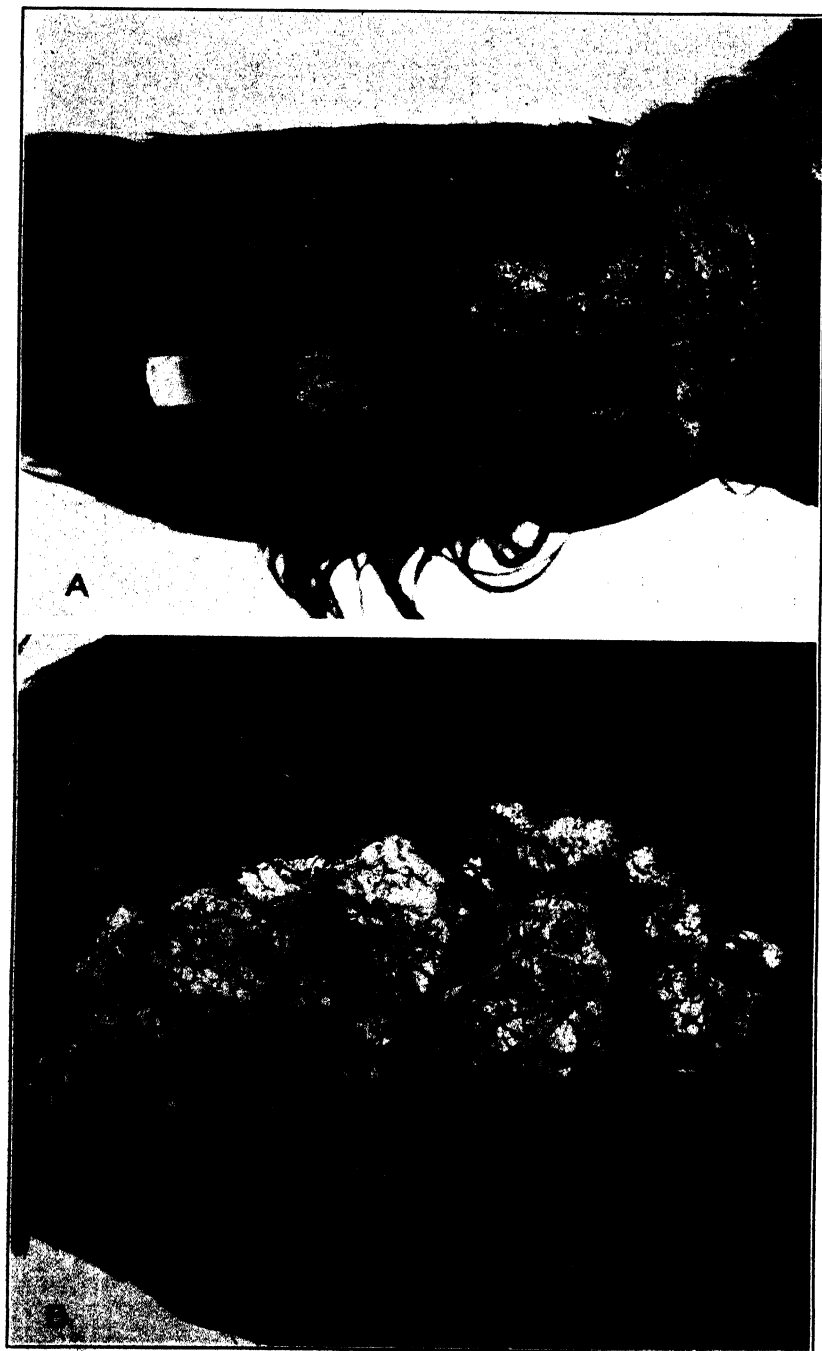


FIGURE 2.—Experimental warts produced on calf 1403 by inoculation. A, Warts on ear; B, well-developed growth at site of inoculation on neck. (Natural size)



FIGURE 3.—Experimental warts on the neck of calf 1402 produced by inoculation with wart filtrate.
(Natural size)

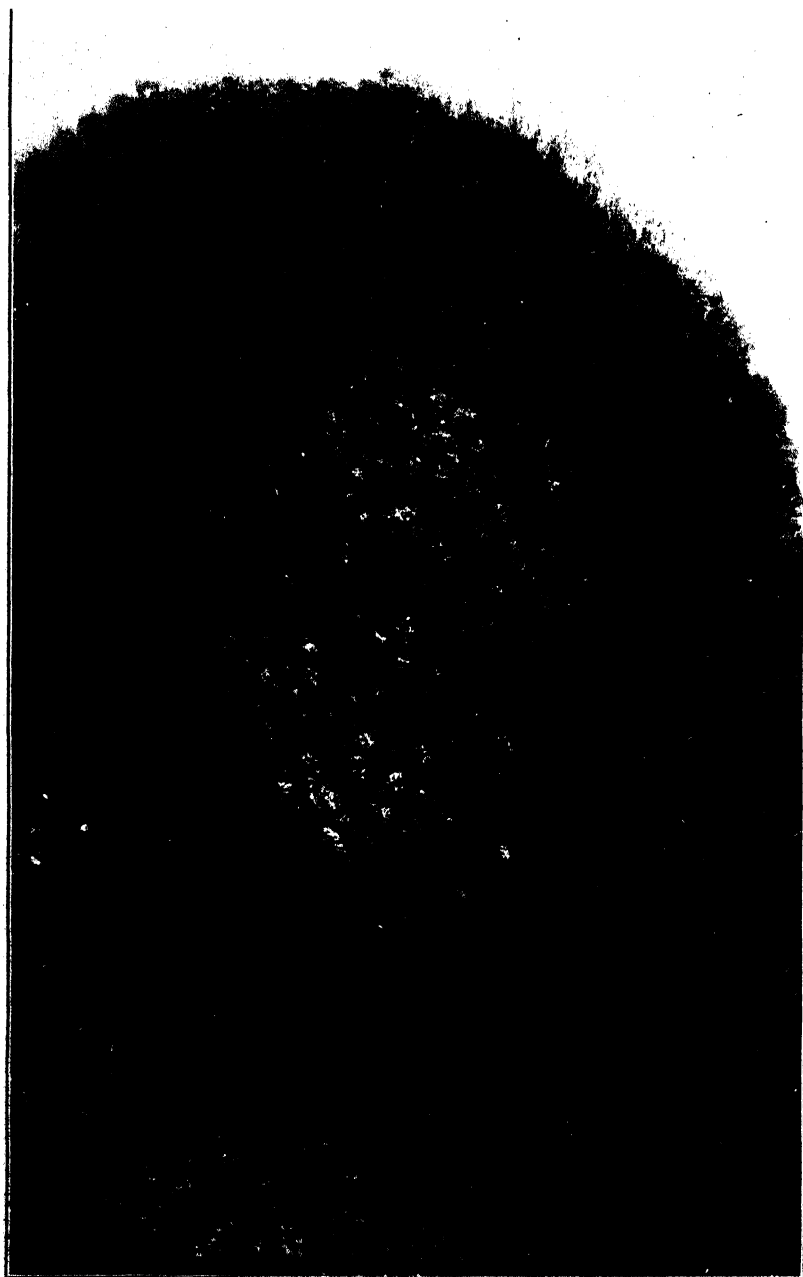


FIGURE 4.—Second generation of filtrate warts on neck of calf 1428, which was inoculated with filtrate of experimental warts from calf 1402. (Natural size)



FIGURE 5.—Section of wart, specimen CH 1, showing typical structure of bovine warts used in original inoculation. $\times 80$



FIGURE 6.—Section of experimental wart from calf 1403, produced by inoculation with unfiltered wart material. $\times 50$

After successfully producing experimental warts with sterile wart filtrates the writer saw no practical value in continuing bacteriological studies of such growths.

A histological comparison of warts from cases 1402 and 1403 shows some rather interesting differences. (Figs. 7 and 8.) In case 1402 there is a marked proliferation of the fibrous structure, the vessels are more numerous, and there is a very noticeable hyperkeratosis. The epithelial tissue is rather limited in this case.

Case 1403 shows typical hypertrophy of the papillae, the increase in the epithelial tissue being very noticeable. The cornified epithelium, it will be noted, is rather scant in this case. The histological variations noted in these two cases are rather interesting but not easily explained.



FIGURE 7.—Section of experimental wart from calf 1402, produced by inoculation with wart filtrate. $\times 40$

While it is generally conceded that warts may be transmitted through experimental inoculations, there are some who question the infectious nature of these growths. Ewing (2) states: "There are numerous clinical observations suggesting the contagiousness of warts, but on analysis they can be dismissed as examples of the transfer of peculiar, chemical irritants, or exposure to the same irritant, or the actual transfer of proliferating epithelial cells." The possibility of producing warty growths by transplanting proliferating epithelial cells is, of course, entirely eliminated in those cases of experimental warts produced by inoculations with filtrates as shown in Table 2.

That any possible chemical substance causing an original wart should remain sufficiently toxic or irritating, after being greatly diluted, subjected to the filtering process, etc., prior to each inoculation, to cause definite warty growths, seems to the writer to be somewhat questionable, especially so when the second generation of such growths is produced.

On the other hand, Wile and Kingery (?) are of the opinion that "the clinical evidence in favor of an infectious agent in the causation of warts is extremely suggestive," and add that the appearance of



FIGURE 8.—Another section of experimental wart from calf 1402 showing a histological structure slightly different from that shown in Figure 7. $\times 40$

the so-called daughter warts following larger warts, the appearance of warts on contiguous surfaces, and the occurrence of groups of individual warts lend color to this view of the contagious nature of common warts. The results of the present experiments seem to be more in accord with the view of the last-mentioned investigators, and tend to substantiate the belief that the causative agent in common warts of cattle partakes of the nature of a filterable virus.

SUMMARY AND CONCLUSIONS

Definite positive results were obtained in 15 out of a total of 22 cattle inoculated with filtered or unfiltered wart material. Eight of the calves giving positive results were inoculated with the unfiltered

wart material and seven with the wart filtrates. The results obtained indicate that common warts in cattle can be experimentally transmitted to bovines under 1 year of age with a fair degree of regularity.

It was also demonstrated that filtrates of bovine warts, proved by cultural tests to be free from cultivable microorganisms, were capable of producing papillomata when inoculated intracutaneously into healthy cattle, and that warts produced by filtrate inoculations may be successfully transmitted in the second generation.

The results of the filtrate experiments seem to justify the conclusion that the causative factor in common warts of cattle is probably of the nature of a filterable virus.

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CHANGES IN THE PECTIC CONSTITUENTS OF APPLES IN RELATION TO SOFTENING¹

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INTRODUCTION

One of the principal changes that takes place in apples as they ripen on the tree and in storage is the softening of the flesh of the fruit. In recent years many studies have been made on the ripening and storage of apples (10),³ and in these the softening of the fruit has been given major consideration. In general, it has been found that there is a gradual, though somewhat irregular, softening of the fruit on the tree as it becomes mature. This softening continues in storage and is the outstanding change that takes place during the process of ripening, the fruit changing from the hard unripe condition to the relatively soft, eating-ripe condition, and finally to the very soft, mealy, overripe condition. The rate at which softening takes place in storage depends primarily upon the temperature at which the apples are kept. For a number of varieties the rate of softening at 40° F. was found to be slightly more than double that at 32°, whereas that at 50° was slightly less than double that at 40°, and that at 60° was nearly double the rate at 50°.

In these studies (10) a fruit pressure tester (9) was used to measure the firmness of the apples. This tester records the pressure in pounds necessary to force a plunger seven-sixteenths of an inch in diameter into the fruit a distance of five-sixteenths of an inch. In penetrating the fruit the tester crushes some of the cells and separates and pushes aside others. The firmness of the apples as measured by the pressure tester is probably due principally to three factors—the size of the cells and intercellular spaces, the thickness of the cell walls, and the ease with which the cells separate. The last two factors are of particular interest in relation to the studies herein reported, as the thickness of the cell walls depends on the thickness of the cellulose or substance of which they are composed and upon the thickness of the layer of cementing substance or pectic material of the middle lamella, while the ease with which the cells separate depends upon the amount of pectic material cementing them together. All three factors might play a part in the softening of the fruit on the tree. In storage, however, there would be no further increase in the size of the cells and intercellular spaces, and softening should be due to changes in the thickness of the cell wall and the ease with which the cells separate.

The studies reported in this paper were undertaken to obtain a better understanding of the underlying chemical changes that take place in apples during the softening process.

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³ Reference is made by number (italic) to "Literature cited," p. 746.

REVIEW OF LITERATURE

In the recent literature dealing with the pectic material in fruit, particularly in apples, the following pectic substances have been recognized: Pectin, which is water soluble and is derived from protopectin probably by enzyme action; protopectin or pectose, which is water insoluble; and the pectic substance of the middle lamella, which is also water insoluble. The total pectic substances really include all three of these, but in this paper only the pectin and protopectin are included as the pectic substance of the middle lamella was not determined.

Several investigators have found softening in fruit to be associated with changes in the pectic material in the cell walls and middle lamella. Carré (3, 4) made extensive studies of the pectic materials of apples in storage. She associated ripening in storage with an increase in pectin and a corresponding decrease in protopectin (pectose), the total pectic materials remaining constant. In the latter stages of storage in which the fruit becomes mealy and breaks down she observed a breaking down of what she termed the "pectic substance of the middle lamella." She found that during this period there is a temporary increase in protopectin, which again decreases. These changes were observed to take place more rapidly at higher temperatures. Similar results were observed by Carré and Horne (6) when microchemical methods were used. With peaches, Appleman and Conrad (1) found that there was a decrease in protopectin and a corresponding increase in pectin with ripening in storage, the rate of these changes increasing with the temperature. When the fruit ripened on the tree very little change was noted in the pectic materials. Emmett (8), working with pears, obtained similar results. At 1° C. the development of pectin was greatly retarded, as was also the softening of the fruit. Appleman and Conrad (2) found an increase in pectin and a decrease in protopectin with increased maturity of tomatoes while on the vine. In canned tomatoes a close relationship existed between the amount of disintegration and the ratio of pectin to protopectin.

From the investigations discussed above it would seem that the cell walls are thickened and cemented together by an insoluble pectic material, usually protopectin. As softening takes place the protopectin is changed to a soluble form, thus permitting the cells to separate readily and decreasing the thickness of their walls. These changes are accelerated by an increase in temperature.

In the investigations previously cited no measurements were made of the firmness of the fruit, except with tomatoes (2), under the various conditions of the experiments. It was the purpose of the present investigation to determine to what extent the changes in the pectic materials of apples are responsible for the softening of the fruit as measured by the pressure tester.

METHODS

The determination of the pectic materials (pectin, total pectic material, and protopectin) was carried out according to the method described by Carré and Haynes (4, 5) with some modifications.

A composite sample of at least 20 apples was used for each determination. The firmness of the fruit was first determined by means

of the pressure tester. The fruit was then pared and cored, and the flesh was run through a sampling press (7) which macerated the tissue to a fine pulp.

The soluble pectin was determined in two ways, as follows:

1. Samples of 100 gm. of pulp were frozen overnight. After thawing, the juice was pressed out through muslin. The pulp was then repeatedly washed with cold water until no appreciable amount of pectin remained. Preliminary investigations had shown that seven washings with 150 to 200 milliliters of water were sufficient to accomplish this. The juice and the first washing were combined, as were also each additional two washings. They were then brought to a boil, cooled, and made to a 500-ml. volume. After filtering, aliquots of sufficient size to yield if possible 0.02 to 0.03 gm. of calcium pectate were transferred to beakers, diluted to 300 ml., made alkaline with 100 ml. of 0.1 normal sodium hydroxide, and allowed to stand overnight. They were then acidified with 50 ml. of normal acetic acid, and the calcium pectate was precipitated by the addition of 50 ml. of molar calcium chloride. The precipitate was then brought to a boil, filtered, and washed with hot water until free from chlorides. The precipitate was washed back into the beaker, boiled, filtered again, and washed. It was then washed into a small tared beaker, dried, and weighed.

2. Portions of the pulp were frozen overnight. After thawing, the juice was pressed out through muslin. Definite volumes, 100 ml. of the juice were brought to a boil, cooled, and made to a 500-ml. volume. After filtering, aliquots of sufficient size to yield 0.02 to 0.03 gm. of calcium pectate were used, and from this point the determinations were carried on as in the first method.

In Tables 1 and 2 the two methods are compared. The soluble pectin in the pulp was obtained by the first method, while that in the juice was obtained by the second. As the results by both methods were practically the same, the second method, which was much shorter, was used in the later determinations.

The determination of the total pectic substances was carried on as follows: The juice from 100-gm. samples of pulp was pressed out through muslin, and the pulp was washed with cold water to remove the organic acids and most of the soluble pectin. The juice and the wash water were combined, brought to a boil, cooled, and made to volume. The residue was then transferred to a flask, covered with 1/75 normal hydrochloric acid, and boiled for an hour under a reflux condenser. The solution was then pressed out through muslin and made to a 500-ml. volume. Extraction of the residue with 1/75 normal hydrochloric acid was repeated twice. After filtering, aliquots of each extraction of sufficient size to yield, if possible, 0.02 to 0.03 gm. of calcium pectate were used, and the determinations were carried on from this point as for soluble pectin.

Protopectin was obtained by subtracting the soluble pectin from the total pectic substance.

EXPERIMENTAL DATA

SOFTENING ON THE TREE IN RELATION TO PECTIC CHANGES

The data on the relation of softening of fruit while on the tree to the changes in the pectic constituents are presented in Table 1 and Figure 1. The amount of soluble pectin in apples as they ripen

on the tree is very small and remains practically constant. As the fruit softens there is a gradual decrease in the percentage of protopectin and a corresponding decrease in total pectin in the Ben Davis apples. In the Jonathan, however, the pectic changes during the first period are negligible even though there is a softening of 6.5 pounds. During the second period the changes correspond to those in the Ben Davis apples. Although the percentage of protopectin decreases, it is probable that the actual amount does not decrease but seems to be less because of the increased size of the fruit. Softening on the tree appears to be associated in part with the apparent decrease in the protopectin content. Other factors, such as increase in the size of the cells and intercellular spaces, may be the principal ones responsible for softening in the rapidly growing fruit.

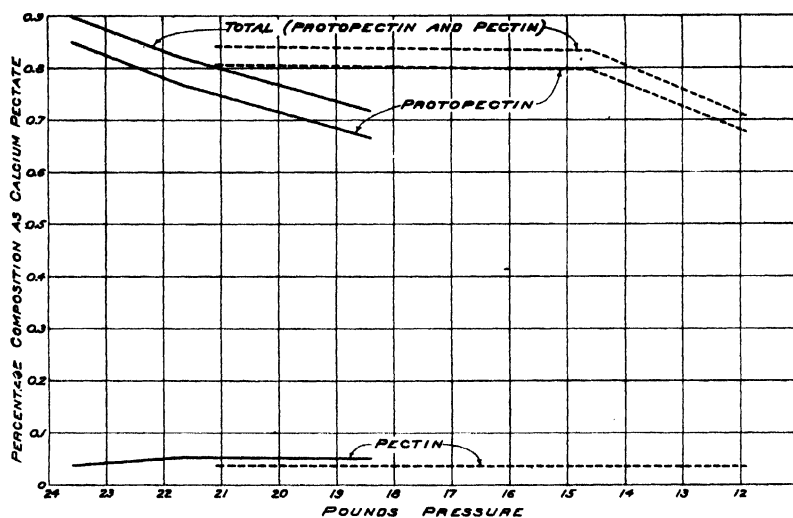


FIGURE 1.—Pectic changes during softening of apples on the tree. Solid line represents Ben Davis in 1926; broken line, Jonathan in 1927

TABLE 1.—Softening of apples on the tree in relation to changes in pectic constituents

[Pectic substances expressed as calcium pectate in percentage of fresh weight]

Variety of apple and date of test	Pressure test	Soluble pectin		Proto-pectin	Total pectic substances
		In juice	In pulp		
Ben Davis:	Pounds	Per cent	Per cent	Per cent	Per cent
Sept. 1, 1926	23.6	0.088	0.048	0.851	0.899
Sept. 17, 1926	21.7	.052	.053	.765	.818
Oct. 14, 1926	18.4	.051	.047	.668	.715
Jonathan:					
July 22, 1927	21.1	.033807	.840
Aug. 22, 1927	14.6	.037796	.833
Sept. 14, 1927	11.9	.034673	.707

SOFTENING IN STORAGE AT 32° F. IN RELATION TO PECTIC CHANGES

Ben Davis, Winesap, and two pickings of Jonathan were used to determine the relation of softening in storage at 32° F. to pectic changes. As softening took place there was a gradual increase in total pectic materials (Table 2 and fig. 2) in Ben Davis and Winesap apples, whereas in Jonathan the total pectic materials remained fairly constant but showed a slight decrease. There was a gradual decrease in protopectin with softening in all varieties except for the last determination on Ben Davis and Winesap apples, when there was an increase. These increases may have been due in part to loss of water by the fruit, which showed considerable shriveling. It is more probable, however, that they were due to the formation of protopectin from the so-called pectic substance of the middle lamella

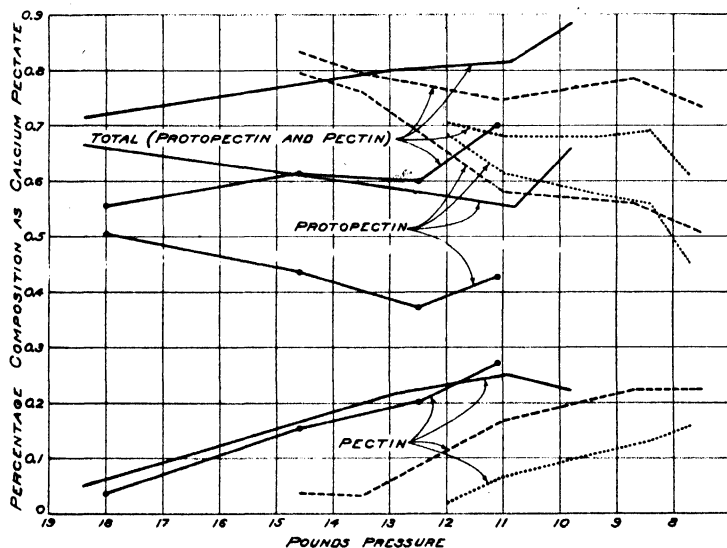


FIGURE 2.—Pectic changes and softening of apples in storage at 32° F. Broken line represents Jonathan I in 1927; dotted line, Jonathan II in 1927; solid line, Ben Davis in 1926; dot-solid line, Winesap in 1926

described by Carré, as the increases in both protopectin and total pectic substances were greater than would be expected, from loss of water, especially as there was no corresponding increase in pectin.

Fruit stored at 32° F. showed a regular increase in soluble pectin, due principally to the hydrolysis of protopectin. In contrast, fruit allowed to ripen on the tree showed no change in soluble pectin during the softening process.

There was no relation between the firmness of the three varieties and their pectic constituents. Jonathan, which was much softer when picked than Winesap and Ben Davis, had a higher protopectin and total pectin content.

The total pectic material and soluble pectin were at all times lower in the later picking of Jonathan than in the earlier picking, while the protopectin was nearly equal in the two pickings at the various stages of softening.

TABLE 2.—*Softening of apples in storage in relation to changes in pectic constituents*
 [Pectic substances expressed as calcium pectate in percentage of fresh weight]

Variety of apple and date of test	Temperature	Pressure test	Soluble pectin		Proto-pectin	Total pectic substances
			In juice	In pulp		
Ben Davis:	° F.	Pounds	Per cent	Per cent	Per cent	Per cent
Oct. 14, 1926 *	32	18.4	0.051	0.047	0.068	0.715
Jan. 31, 1927		12.9	.216	.214	.587	.801
Apr. 6, 1927		10.9	.250	.262	.552	.814
July 18, 1927		9.8	.221	.228	.656	.884
Winesaps:						
Oct. 27, 1926 *	32	18.0	.036	.090	.504	.564
Feb. 14, 1927		14.6	.154	.175	.436	.611
Apr. 22, 1927		12.5	.202	.231	.371	.602
July 22, 1927		11.1	.263	.275	.425	.700
Jonathan I:						
Aug. 22, 1927 *	32	14.6	.037796	.833
Oct. 3, 1927		13.5	.032760	.792
Nov. 14, 1927		11.0	.166579	.745
Dec. 21, 1927		8.7	.223561	.784
Mar. 1, 1928		7.5	.225508	.733
Jonathan II:						
Sept. 14, 1927 *	32	11.9	.034673	.707
Oct. 14, 1927		12.0	.018687	.705
Nov. 16, 1927		11.0	.063618	.681
Dec. 21, 1927		9.3	.107574	.681
Mar. 3, 1928		8.4	.130560	.690
May 2, 1928		7.7	.158452	.610
Jonathan I:						
Aug. 22, 1927 *	40	14.6	.037796	.833
Sept. 15, 1927		13.5	.055705	.760
Oct. 5, 1927		9.7	.159627	.786
Oct. 24, 1927		8.5	.229447	.676
Dec. 17, 1927		7.7	.252435	.687
Jonathan I:						
Aug. 22, 1927 *	50	14.6	.037796	.833
Sept. 8, 1927		13.2	.048737	.785
Sept. 26, 1927		8.7	.237454	.691
Oct. 26, 1927		7.2	.278384	.662
Jonathan I:						
Aug. 22, 1927 *	60	14.6	.037796	.833
Aug. 26, 1927		14.8	.015730	.745
Aug. 31, 1927		14.2	.017681	.698
Sept. 9, 1927		9.3	.185549	.734
Sept. 30, 1927		7.5	.270408	.678

* Date picked and placed in storage.

SOFTENING IN STORAGE AT VARIOUS TEMPERATURES IN RELATION TO PECTIC CHANGES

Fruit from the second picking of Jonathan was placed in storage at 32°, 40°, 50°, and 60° F. Determinations were made at intervals, and the data are presented in Table 2 and Figure 3.

There was some irregularity in the percentage of total pectic substances and protopectin at the various temperatures. In general, there was a tendency for the amount of total pectic substances to decrease slightly with softening. There was a decrease in protopectin with softening, as shown in Figure 3, from more than 0.8 per cent at the start with a pressure test of 14.6 pounds to slightly less than 0.45 per cent at 40°, 50°, and 60°, and 0.53 per cent at 32° F., when the fruit tested 8 pounds. A very consistent increase in soluble pectin occurred at all temperatures, with softening from 0.04 per cent at the start to about 0.25 per cent when the fruit tested 8 pounds.

That the rate of softening is proportional to the rate at which the protopectin is converted into pectin is shown in Table 3. In storage at 32° F., 100 days were required for the fruit to soften from a pressure test of 14.6 pounds, the hard-ripe condition at picking time, to 10

pounds, which represents a full-ripe condition. At the same time the protopectin decreased from 0.796 to 0.585 per cent and the pectin increased from 0.037 to 0.195 per cent. Using the conditions found at 32° storage as standards for comparison with those at the other temperatures, it was found that at 40° it required 42 days to reach the same condition of softness, and 44 and 51 days, respectively, for the corresponding changes in the protopectin and pectin to take place. At 50° it required 29 days for the pressure test to become 10 pounds, and 27 and 31 days, respectively, to reach 0.585 per cent protopectin and 0.195 per cent pectin, while at 60° the corresponding changes took place in 16, 15, and 19 days, respectively.

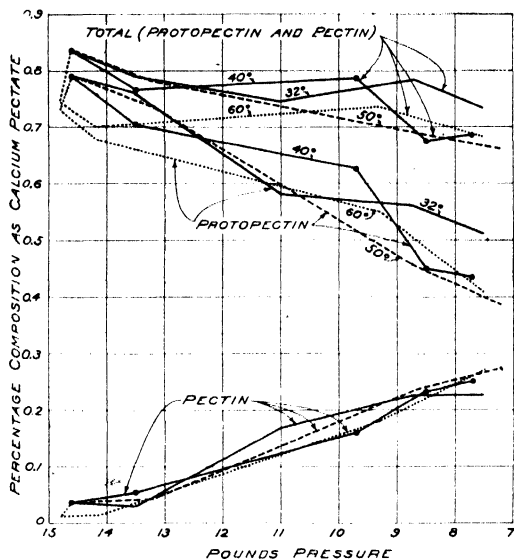


FIGURE 3.—Pectic changes and softening of Jonathan apples in storage at different temperatures. Solid line represents 32° F.; circle-solid line, 40°; broken line, 50°; dotted line, 60°

TABLE 3.—Comparison of rate of softening of apples in storage with rate of change in percentage of protopectin and pectin at different temperatures

Temperature		Days required to reach—		
°F.	°C.	Pressure test of 10 pounds	Proto-pectin content of 0.585 per cent	Pectin content of 0.195 per cent
32	0	100	100	100
40	4.4	42	44	51
50	10.0	29	27	31
60	15.5	16	15	19

At the time the first determination was made on the fruit stored at 60° F. there was a slight increase in firmness which was associated with a slight decrease in pectin and a considerable decrease in protopectin and total pectic substances. With this exception, the changes in the pectic constituents at the various temperatures were proportional to the rate of softening at each temperature.

SUMMARY AND CONCLUSIONS

The relation between the softening of the fruit on the tree and in storage, as measured by a mechanical pressure tester, and changes in the pectic constituents of the fruit was studied, and from the data obtained the following conclusions seem justified:

Softening on the tree as apples approach maturity is to some extent associated with a decrease in the percentage of protopectin and a corresponding decrease in total pectic substances, whereas the pectin, which is present in very small amounts, remain constant. Softening on the tree can not be entirely accounted for by changes in the pectic constituents.

The relative firmness of different varieties of apple likewise can not be accounted for by differences in their pectic constituents.

Softening in storage is apparently due to the conversion of insoluble pectic substances, principally protopectin, into soluble form. The rate of conversion at different temperatures is proportional to the rate of softening.

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THE TENDENCY OF THE CROWN-GALL ORGANISM TO PRODUCE ROOTS IN CONJUNCTION WITH TUMORS¹

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INTRODUCTION

In his early work with the crown-gall organism, *Bacterium tumefaciens*, Erwin F. Smith made definite reference to the production of roots by the apple strain of that organism (20).² Later, when writing about the analogy of plant to animal cancer through the formation of teratomata (17, 18), he described and pictured many leafy shoots on or along the sides of tumors produced by inoculating tobacco, Bryophyllum, and geranium with several different strains of the organism. He attributed these abnormal leafy shoots, flower buds, and root formations to the kind of tissues inoculated, together with the growth conditions of the plant at the time of inoculation, and he thought that the diffusible products of the bacteria within a growing tumor could stimulate surrounding uninoculated tissues to multiply. His interest in showing the analogy of plant to animal tumors influenced him to work along the more striking line of shoot production.

The stimulation to root production by the various strains of *Bacterium tumefaciens* has been somewhat overlooked. This statement does not refer to the apple strain, however, which has been recognized since 1908 as a strain that produces a mass of roots (fig. 1) when it enters a wounded or poorly grafted apple root. Some investigators maintain that there are two types of apple strain, one that produces only tumors and another that produces only masses of roots. In the writer's opinion, which is based on years of observation and investigation, there is only one apple strain, and in this paper, which deals largely with the apple strain, the crown gall and infectious hairy root of apple are considered as the same disease, which manifests itself as a tumor, a mass of roots, or a combination of both. It happens that in those cases where roots occur in clumps without a definite tumor, inconspicuous swellings are usually present.

REVIEW OF LITERATURE

In his work with crown gall, which embraced hundreds of experiments in orchards and nurseries, Hedcock (3) in 1905 established the pathological effect of crown gall on orchard trees. He produced many cross infections with minced galls from peach, apricot, almond, apple, chestnut, and other trees by inserting pieces in wounds on seedling trees planted in sterile soil, and found that by this method he could produce infection readily on some hosts. The apple, however, was infected with difficulty, and when galls of the apple were transferred to other fruit trees infection occurred only on a limited

¹ Received for publication Apr. 1, 1929; issued November, 1929.

² Reference is made by number (italico) to "Literature cited," p. 765.



FIGURE 1.—Hairy root on Windsor apple from Nebraska. Photographed November, 1927. One-half natural size

number of hosts (4). Hedgcock noted a marked difference in the susceptibility of different varieties of apple trees to crown gall and he found that infection on some varieties tended to the gall type and on others to the hairy-root type. He did not establish the cause of these outgrowths on fruit trees. He reported that not all the malformations on apple trees were due to an infectious agent, but that many were caused by poor grafting unions which resulted in an excess of callus formation (5).

In 1907 the bacterial origin of the crown gall on the Paris daisy was established by Smith and Townsend (19), and the causal organism was named *Bacterium tumefaciens*. In 1911 it was further determined that the organism had the ability to cause tumor growths on plants in many unrelated families. One of these hosts was the apple, on which the disease was manifested by a definite gall or a small outgrowth from which a mass of roots projected (20).

The malformations on apple stems reported years ago in England are considered by horticulturists there and by Charles F. Swingle in the United States as varietal characteristics. They have been designated as burr knots (2, 8, 21).

The writer made many attempts to isolate *Bacterium tumefaciens* from burr knots on apple but without success (1). Undoubtedly there are outgrowths on apple stems caused by *Bact. tumefaciens*, but the writer believes that the burr knot or stem tumor of apple is produced by some other agent.

Studies of outgrowths on grafted apple trees and proof of the production of callus formation without the intervention of *Bacterium tumefaciens*, and other related crown-gall investigations, have been carried on by Riker, Keitt, Melhus, and Muncie, working alone or in groups (6, 7, 9, 10, 11, 12, 13, 14). They induced overgrowths on grafted apple trees at the scion tips, using aseptical methods with the stock and scion cuttings to reduce as far as practicable or completely eliminate the presence of any organism. In their isolation studies with apple galls they obtained what appeared to be *Bact. tumefaciens* colonies on poured plates, but except in a very few cases inoculations with subcultures failed to produce the outgrowths necessary for proof. The percentages ran low even when isolations were made from 100 to 200 and more apple galls. The plants used for testing the pathogenicity of these isolations were mostly tomato, but tobacco and geranium were also used. These three are all hosts on which it is difficult to produce tumors with the apple strain, although tumors are produced readily on them with some other crown-gall strains. In fact, there is no quick way of testing the pathogenicity of *Bact. tumefaciens* isolated from apple crown gall. The Paris daisy, *Impatiens balsamina*, and string-bean stems have been found by the writer, as noted elsewhere in this paper, to become infected with the apple strain in less time than is required to produce outgrowths on the apple stems, but usually three to seven weeks must be allowed even for these hosts.

Recently Siegler (16) also isolated *Bacterium tumefaciens* from galls on apple trees and proved its pathogenicity by producing roots and outgrowths on apple and various other hosts. His isolations also showed the slowness and inadequacy of the apple strain in producing definite roots and sizable tumors on tobacco, geranium, and tomato plants.

As shown in some of the publications cited, the malformations on apple trees are classified under several names, the one called noninfectious hairy root being considered by some workers the most prevalent type.

Riker, Banfield, Wright, and Keitt (15) recently isolated from apple crown gall an organism which produced roots when inoculated into apple trees but which failed to produce infection on tomato plants. They inoculated 15 strains isolated from 15 different sources into young apple shoots, and all produced roots; the organism was reisolated from the enlargements at the bases of these roots. This the writer considers the typical apple strain of crown gall, which will not infect tomato, or at least will not produce a conspicuous infection, until it has passed through and been isolated from another host. What Riker and his associate call a root-stimulating organism, as though it were unrelated to crown gall, is the apple strain, which in reality is a root-stimulating organism. The apple strain showed this tendency in 1908 when the writer first isolated it from both galls and hairy roots on apple trees. It possesses this strain difference just as it also possesses similarities to other strains of crown gall, and the writer does not think it should be placed under any new classification.

THE APPLE STRAIN OF BACTERIUM TUMEFACIENS

ROOT-PRODUCING ABILITY

The general tendency of the crown-gall organism isolated from an apple tree naturally infected with hairy root is to produce roots when inoculated into an apple tree (figs. 2, Aa, and 3, B), but it can produce definite tumors (fig. 2, Ab) and some flat outgrowths covered or partially covered with root primordia (fig. 2, D).

This ability of the apple strain to stimulate the production of roots is not confined to infections on the apple tree, for it readily stimulated root production on stems of the Paris daisy (fig. 4, Aa, Ab), Bryophyllum (fig. 5, Ba), and sugar-beet root (fig. 6, A). Not every successful inoculation produces roots, and a tumor may form without roots, as is shown on the Bryophyllum. (Fig. 5, Bb.) Sugar beets inoculated with the apple strain produce roots as the usual type of infection, but frequently there are tumors of various sizes present. Figure 6, B, shows a definite tumor produced on a sugar beet inoculated with the hop strain and, growing in the same bed, a sugar beet inoculated at the same time with the apple strain. (Fig. 6, A.)

Tumors without roots were produced on rose and bean plants by the same culture that produced roots on daisies and sugar beets. Inconspicuous root primordia were present on the tumors, but the surfaces were not covered by them.

An organism isolated from a natural rootless tumor of apple produced roots and definite sizable tumors on Paris daisy stems. (Fig. 5, D and C.) It also produced definite tumors on apple trees. (Fig. 3, C.) An organism isolated from an induced tumor (without roots) on an apple tree can produce both tumors and roots. This was proved by inoculating sugar beets with such an isolation. Masses of roots occurred at the inoculation places on eight of them, and in addition tumors occurred on four.

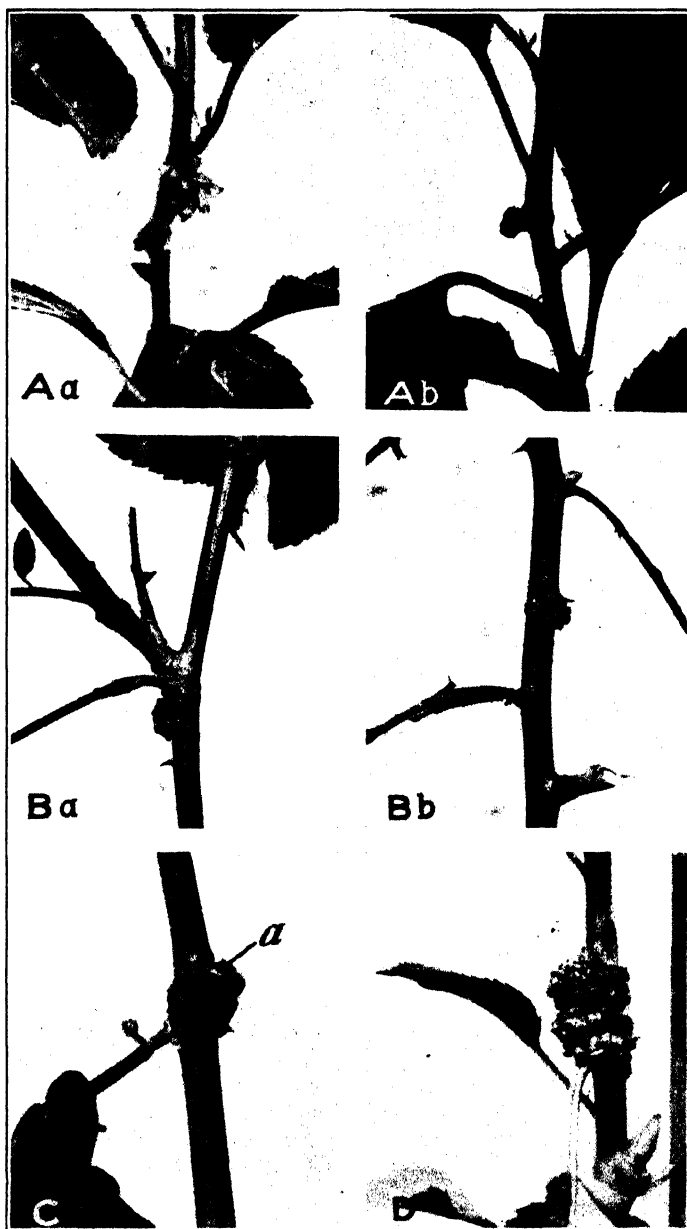


FIGURE 2.—Inoculations of various hosts with the organism isolated from an apple hairy root. All natural size: A, Seedling apple trees, two months after inoculation; Aa, tumor with roots; Ab, tumor, without roots. Ba and Bb, Rose stems, five months after inoculation. C, Stem of string bean, three months after inoculation. Small white root, *a*, developed after plant had been kept in refrigerator at 10° to 14° C. for 20 hours. D, Seedling apple tree, seven months after inoculation



FIGURE 3.—A, The peach strain of *Bacterium tumefaciens* inoculated into a growing stem of an apple tree. Time, five months. Three-fourths natural size. B, Apple tree inoculated at crown with organism isolated from the hairy-root type of infection, showing hard tumor and masses of roots. Time, nearly five months. Reduced in size. C, Apple tree inoculated at crown with organism isolated from tumor on apple without the root masses, and in this case producing only tumors. Time, two months. Reduced in size. The same isolation produced masses of root primordia on Paris daisy stems (fig. 5, D) and a definite tumor with roots on Paris daisy at crown (fig. 5, C)

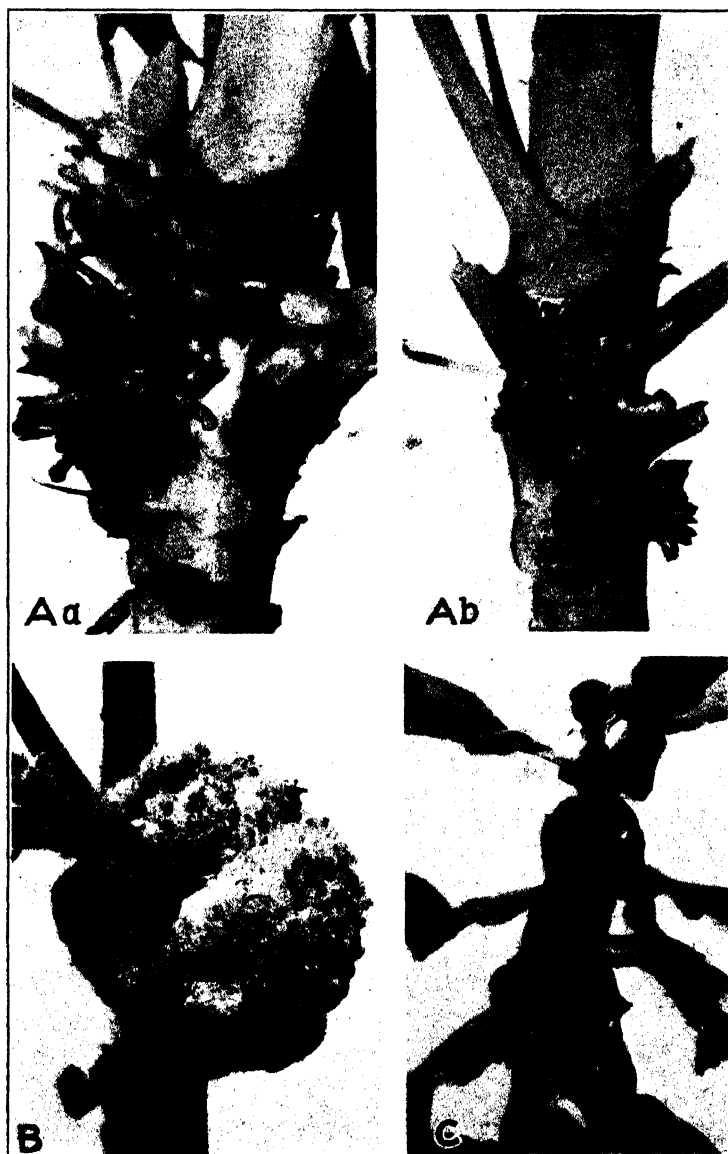


FIGURE 4.—Aa, and Ab, Paris daisy stems inoculated with the organism isolated from hairy root of Windsor apple shown in Figure 1, November 23, 1927. Photographed March 3, 1928. \times nearly 2. B, Tobacco stem inoculated with rose strain of *Bacterium tumefaciens* showing tumor with root primordia. Time, two months. \times nearly 2. C, Tobacco stem inoculated with peach strain of *Bact. tumefaciens* showing tumor with roots. Time two months. Natural size

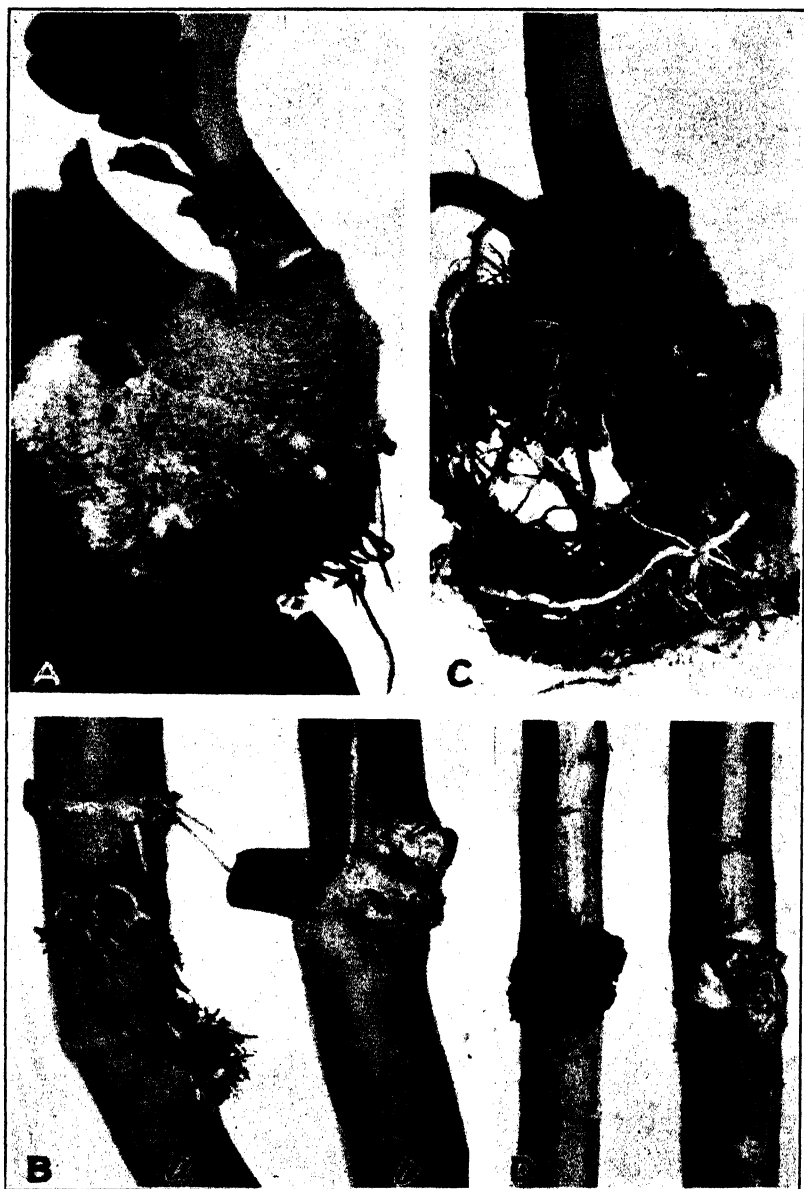


FIGURE 5.—A, *Bryophyllum pinnatum* inoculated with the hop strain of *Bacterium tumefaciens*, showing tumor and roots. Time, three months. \times nearly 5. B, *Bryophyllum pinnatum* inoculated with the apple hairy-root strain of *Bact. tumefaciens*. The stem with the tumor and no definite roots, Bb, was inoculated with the original isolation colony. The stem with roots, Ba, was inoculated with a reisolation colony on the same date. Time of both, five months. Natural size. C, Tumor with roots on Paris daisy produced by inoculating the crown with an organism isolated from a definite tumor on apple (in distinction from hairy root). Time, 10 months. Natural size. Figure 3, C, shows the same isolation inoculated into apple crown. D, Tumor and masses of root primordia produced by inoculating stems of Paris daisy with the same organism used to produce C. Time, eight months. Natural size



FIGURE 6.—A, Sugar beet inoculated with the apple hairy-root strain of *Bacterium tumefaciens*, March 27, 1928. Photographed June 23, 1928. Tumors and roots produced. B, Sugar beet inoculated with the hop strain at the same time and photographed on the same date as A. Only a tumor produced. Natural size

THE APPLE TREE AS A HOST

The apple tree is a less favorable host to the crown-gall organism than the peach and apricot, on whose tender cortex tumors form more readily. But it is not entirely due to the less tender tissues of apple that tumors or hairy roots form more slowly on it than on peach and apricot. The appearance of tumor growths on rapidly growing apple seedlings inoculated with the apple strain usually requires two months, which is twice as long, or even longer, than the time required for tumors to appear on peach or apricot trees inoculated with the peach and apricot strains of *Bacterium tumefaciens*. Even the active peach strain when inoculated into apple trees is a slow tumor producer, but it is much more rapid than the apple strain. (Fig. 3, A.) The slowness of the peach strain in producing tumors on apple trees may be due to the more acid condition of the apple stem, as indicated by the pH, 4.6. The peach stem has a pH value of 4.8. The tests of both peach and apple stems were made near the close of the summer, however, and there might have been a wider difference in the pH values had the tests been made at some other time of the year.

The slowness of the apple strain in producing tumors on its native host is characteristic of its action on the other hosts to which it is infectious. When young, quickly growing daisy plants are inoculated with the apple strain by using subcultures soon after isolating, the tumors or roots do not appear even as swellings until six or seven weeks have elapsed, and growth continues slowly. Figure 4, Aa and Ab show daisy stems three and one-half months after inoculation with this strain. The peach, daisy, apricot, gooseberry, pecan, honeysuckle, and some other strains show infections on the daisy within a week and sometimes less after inoculation. The hop strain will not produce a tumor on the daisy, although the daisy strain will produce a tumor rather readily on the hop root or stem.

The apple strain isolated from the hairy root illustrated in Figure 1 did not produce so many infections on its natural host as did the peach, hop, and daisy strains on theirs. These last three strains may produce 90 to 100 per cent infection. A weakly pathogenic colony of the apple strain may produce less than 20 per cent infection on apple trees, and an infectious colony 40 to 50 per cent. The same colony may cause 60 to 70 per cent infection (or even more) on Paris daisy and *Impatiens balsamina*. Reisolation colonies from apple-crown gall on daisy produce as high as 80 per cent infection. Ten infectious reisolation colonies obtained from roots on daisy (fig. 4, Aa), when inoculated into 54 daisy stems, produced roots on 9 (fig. 7, A, B, D); tumors on 34, some with root primordia (fig. 7, C); and no infection on the remaining 11.

REACTIONS OF VARIOUS HOSTS TO INOCULATIONS

Young string-bean plants inoculated with the apple hairy-root isolation did not soon produce outgrowths, as four weeks or longer elapsed before definite swellings appeared. Bean plants of the same age inoculated at the same time with the hop strain developed 6-mm. tumors within three weeks. When bean stems were inoculated with the apple hairy-root isolation definite tumors rather than roots or a combination of roots and tumors were produced.

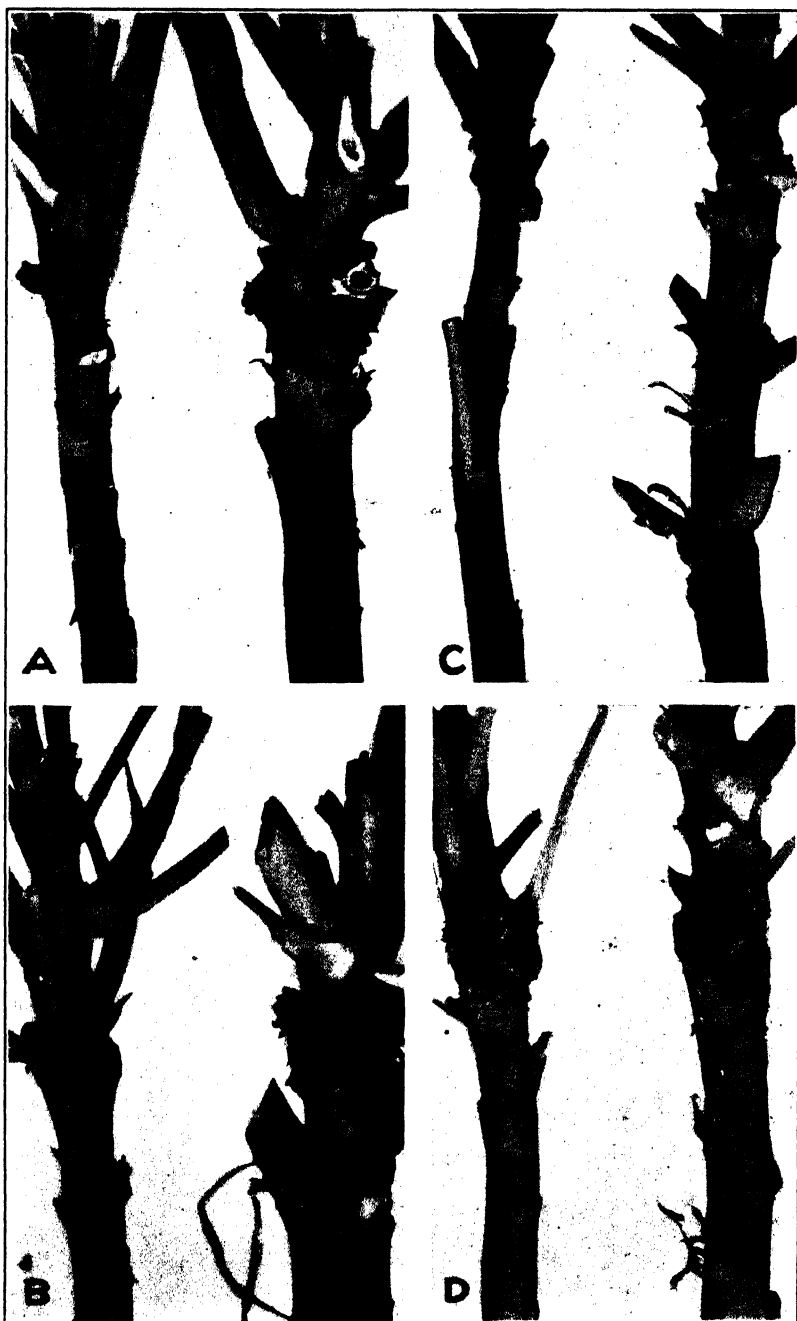


FIGURE 7.—A-D, Paris daisy stems inoculated, March 13, 1928, with four different colonies reisolated from outgrowths shown in Figure 4, Aa. Photographed May 12, 1928. All natural size

When the rose was inoculated with the cultures used for beans and daisies it was slow to show infection, and when very tender shoots were inoculated infection did not appear for six weeks or longer. The type of outgrowth was a tumor with root primordia.

The castor-bean plant (*Ricinus communis*) was thought for some time to be immune to the apple strain. One set of inoculations appeared to be negative after two months. While another set of inoculations was in progress to check up this earlier one, small outgrowths appeared on the first set three months after inoculation, and on the second set between two and three months after inoculation. The tumors never grew larger than 4 mm. across, but they were definite infections which might easily have been overlooked if the controls had not been so definitely without swellings. The percentage of infection was about 50. Some of the *Ricinus* stalks were split longitudinally through the inoculated area, and little masses of tumors were found in the pith extending into the hollow center at these points. To make absolutely certain that the external and internal outgrowths were caused by the organism that had been inserted, platings were made from the tiny outside tumors and from the pith outgrowths, and crown-gall colonies appeared on both sets of plates. Subcultures made from colonies on these two sets of plates produced tumors when inoculated into daisies.

Ricinus is an acid plant with a pH of 5.4, but the peach and apple stems are more acid (pH 4.8 and 4.6, respectively), and the apple strain causes infection on them. Evidently there is some other feature of *Ricinus* than its hydrogen-ion concentration, perhaps a high titratable acidity, which makes it unfavorable as a host. The hop and peach strains, however, found *Ricinus* a very favorable host and produced tumors three-fourths to 1 inch in diameter in four weeks on vigorous plants.

Geranium and tobacco, like *Ricinus*, are favorable hosts to many strains of *Bacterium tumefaciens*, but are only slightly susceptible to the apple strain. For some time these plants were considered immune to the apple strain. Small flat inconspicuous outgrowths (4 to 5 mm. in diameter) of the tumor type were produced only under the most favorable conditions in two to four months, and but a small percentage of the inoculations caused infection (3 out of 24 with geranium and 4 out of 24 with tobacco). No root growths or tumors with roots were produced. Figure 8, D, shows the results of inoculating tobacco with the isolation from the hairy root shown in Figure 1.

DISTINCTIVE FEATURES

Isolations of the apple strain produce fewer virulent colonies, and the strain has a more limited number of host plants than any of the other strains except the rose. Slowness in producing tumors seems to be inherent in the strain itself, for slowness is characteristic of its action on its own host, the apple, as well as on other hosts. Moreover, the organism is slow in its growth in culture media. Like the rose strain, it is less translucent on beef and Thaxter (potato-dextrose) agar than other strains, which makes it appear in some stages quite unlike the crown-gall organism. In no medium used by the writer does it produce such a heavy growth as the other strains of *Bacterium tumefaciens*. Most isolations of the rose strain must be excepted, for this strain likewise failed to produce a heavy growth in the media used.

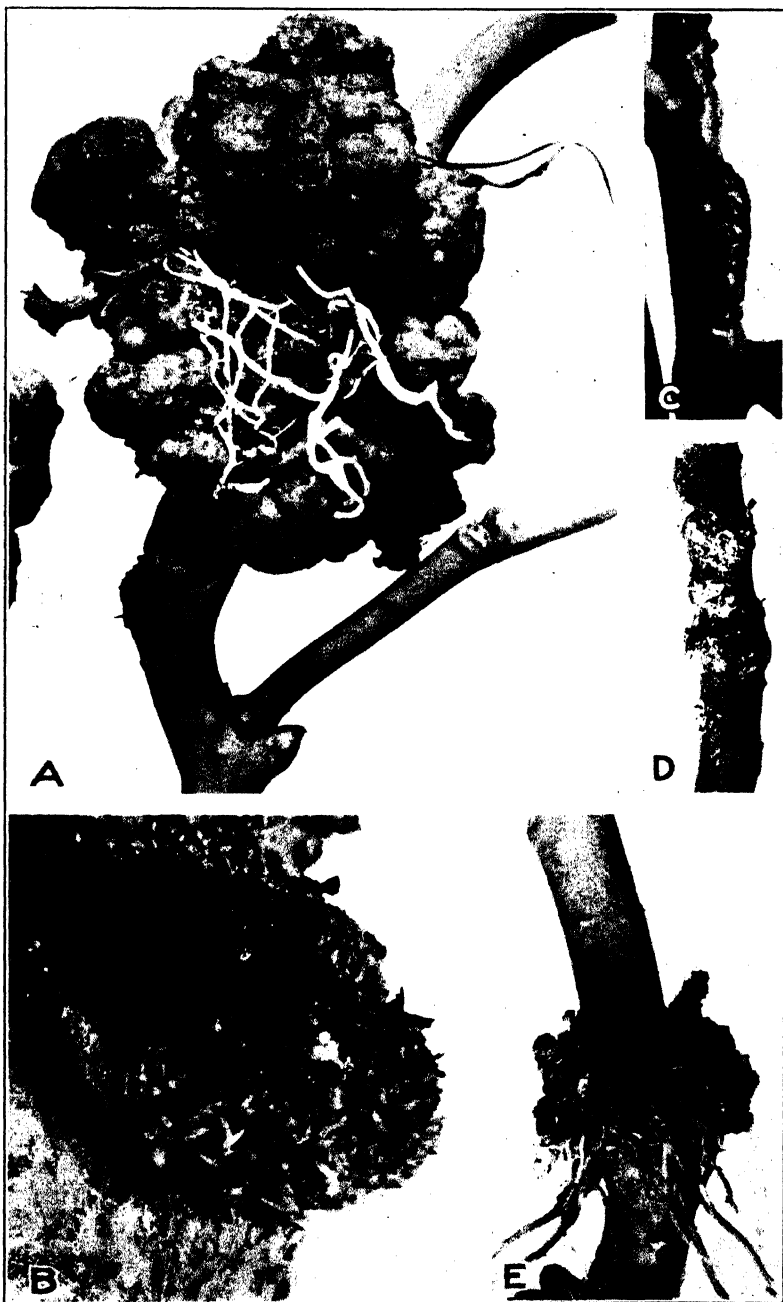


FIGURE 8.—A, Nasturtium stem inoculated with the hop strain of *Bacterium tumefaciens*, showing tumor and roots. Time, three months. Nearly natural size. B, Collards stem inoculated with poplar strain, showing tumor and roots. Time, two months. $\times 2$. C, Tomato stem inoculated with daisy strain, showing tumors with a few root primordia. Time, five weeks. Natural size. D, Tobacco stem inoculated with the apple strain isolated from the apple hairy root shown in Figure 1. Time, five months. Natural size. E, Geranium inoculated with chrysanthemum strain, showing tumor and roots. Time, one year. Natural size

The apple and rose strains are alike (1) in producing comparatively few virulent isolation colonies, (2) in their habit of growth and appearance in culture media, (3) in having a more limited number of host plants than the other strains, and (4) in their slowness to produce tumors, although some tumors eventually become as large as the average tumor produced by other strains.

That the crown-gall organism is able to adapt itself to new conditions was shown by the apple strain in its reisolation colonies. The reisolations were made from apple hairy root produced on daisies. (Fig. 4, A a.) When these reisolation colonies were inoculated into other daisy stems infection appeared sooner than in the original inoculations. While with the original organism it took nearly two months for the inoculations to show infection, with the reisolation colonies it took a few days less than one month. The tendency to produce roots as well as definite tumors was retained in the reisolation colonies. (Fig. 7, A-D.) Of the 10 colonies picked off and tested, all proved infectious to daisy stems, and 80 per cent of the 54 inoculations were positive.

The writer has never been able to produce a definite swelling on tomato plants with the apple strain, although she has made inoculations in top and base and in the neighborhood of adventitious roots and has used plants of various ages and isolations and reisolations from different sources. Thirty-six tomato plants inoculated with the isolation from the hairy root shown in Figure 1 gave negative results. Small inconspicuous swellings with smooth surfaces were produced on tomato stems inoculated with a reisolation from apple tumor on daisy. The tomato stem is a favorable host to the peach and hop strains, but only moderately so to the daisy strain. (Fig. 8, C.) Tobacco is a favorable host to the rose (fig. 4, B), peach (fig. 4, C), and hop strains. If the peach strain of *Bacterium tumefaciens* in the soil should pass through the apple crown or root and produce infection, an isolation from this apple-peach tumor would probably still be able to produce an infection on the tomato stem as the original organism does. In his work with teratomata Smith (18) used the hop strain largely, and he pictured many hop tumors on tobacco.

The cultural characteristics of most of the reisolation colonies changed. The reisolations grew more rapidly and more abundantly in culture media than the original isolations, and on beef and potato-dextrose agar they looked more like the vigorous peach and hop strains than like the original isolations.

Further proof of the adaptability of the crown-gall organism was shown by the reisolation of the apple strain from tumors on *Ricinus*. The inconspicuous outgrowths which took three months to form on *Ricinus* produced reisolation colonies which developed outgrowths on daisy stems more rapidly than the apple-strain reisolations made from the daisy itself. The *Ricinus* reisolations of apple strain when used for inoculations showed good infections in the daisy in two to three weeks, whereas it took the daisy reisolations (apple strain) almost four weeks. Furthermore, the reisolation of the apple strain from *Ricinus*, like the reisolation of the apple strain from daisy, produced colonies which grew much more vigorously in culture media than the original apple isolation colonies, to which they were related.

It might be mentioned here that the strain isolated from a natural tumor on the crown of a hop plant became much more virulent and made a heavier growth in culture media after it was isolated from a hop tumor induced on a sunflower plant.

ROOT PRODUCTION WITH OTHER STRAINS OF *BACTERIUM TUMEFACIENS*

Although the apple alone of all the crown-gall strains seems to possess the inherent ability to produce roots whether it enters the crown, root, or stem of the apple tree or some other susceptible plant, still there are other strains of *Bacterium tumefaciens* that will produce roots on certain plants and under certain conditions. These roots are usually in combination with tumors and are produced mostly on plants that can be propagated easily from cuttings.

The hop strain produces roots very readily on *Impatiens balsamina*, the common garden balsam. The roots grow on or near the tumor and may be from 5 to 25 mm. or more in length. (Fig. 9, B.) The peach and poplar strains also produce roots on *I. balsamina* in less time than it takes the apple strain to do so. (Figs. 9, A, and 10, A-C.) It is very likely that all the strains of *Bacterium tumefaciens* will produce roots on this susceptible host and that each will display some individuality in its root production, as is shown by the four strains pictured.

One month after the balsam stems were inoculated with the hop strain, roots 10 to 12 mm. long had been produced, and the tumors were 1 to 1.5 cm. in diameter, or even larger. The roots on the stems of the plants inoculated with the poplar strain were not so long, and those inoculated with the peach strain were much shorter, being only 3 to 4 mm. In one month the inoculations, made with the original isolation from the apple hairy root and with two reisolations, showed tiny tumor swellings 3 to 4 mm. across with root primordia but no root extensions. The photographs showing root extensions of apple strain were made two months after inoculation. The experiment was repeated, but the apple strain was less active and only a few roots extended from the tumors even after three months. The three other strains showed vigorous roots.

The masses of roots often produced at the crown of chrysanthemum plants and the abnormal number of roots on rooted cuttings of chrysanthemum have been found to be produced in some cases by *Bacterium tumefaciens*. These masses of roots are not beneficial to the plant, for the vigor and substances that should go into the production of leaves and flowers are used in the thickened masses of roots. The organism isolated from chrysanthemum roots produces both roots and tumors when inoculated into susceptible plants. An example of a tumor with roots on a geranium produced by inoculating it with the chrysanthemum strain is shown in Figure 8, E.

The hop strain inoculated into Bryophyllum and nasturtium stems can produce definite tumors with roots. (Figs. 5, A, and 8, A.) So also can the poplar strain inoculated into collards stems. (Figs 8, B.) Occasionally the daisy strain will produce a few roots from the tumors on daisy stems.

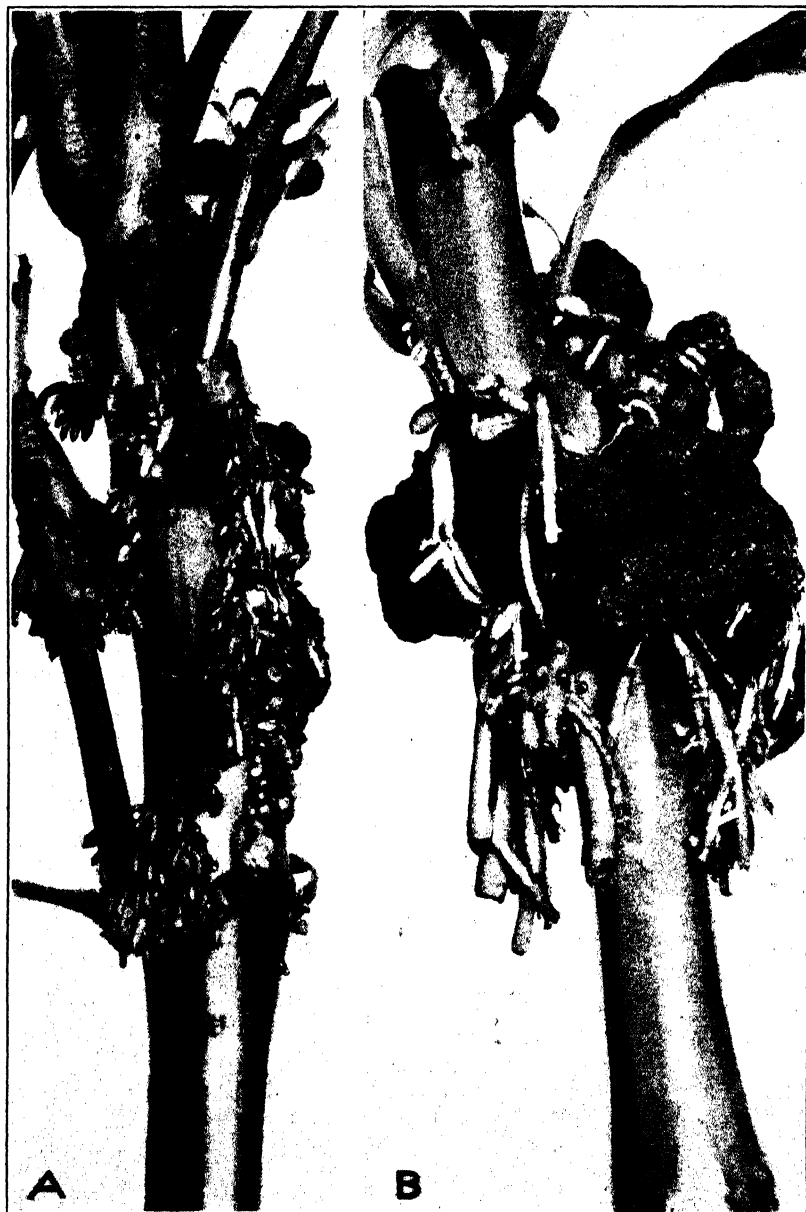


FIGURE 9.—*Impatiens balsamina* inoculated June 15, 1928: A, With peach strain of *Bacterium tumefaciens*; B, with hop strain. Photographed September 17, 1928. Both natural size

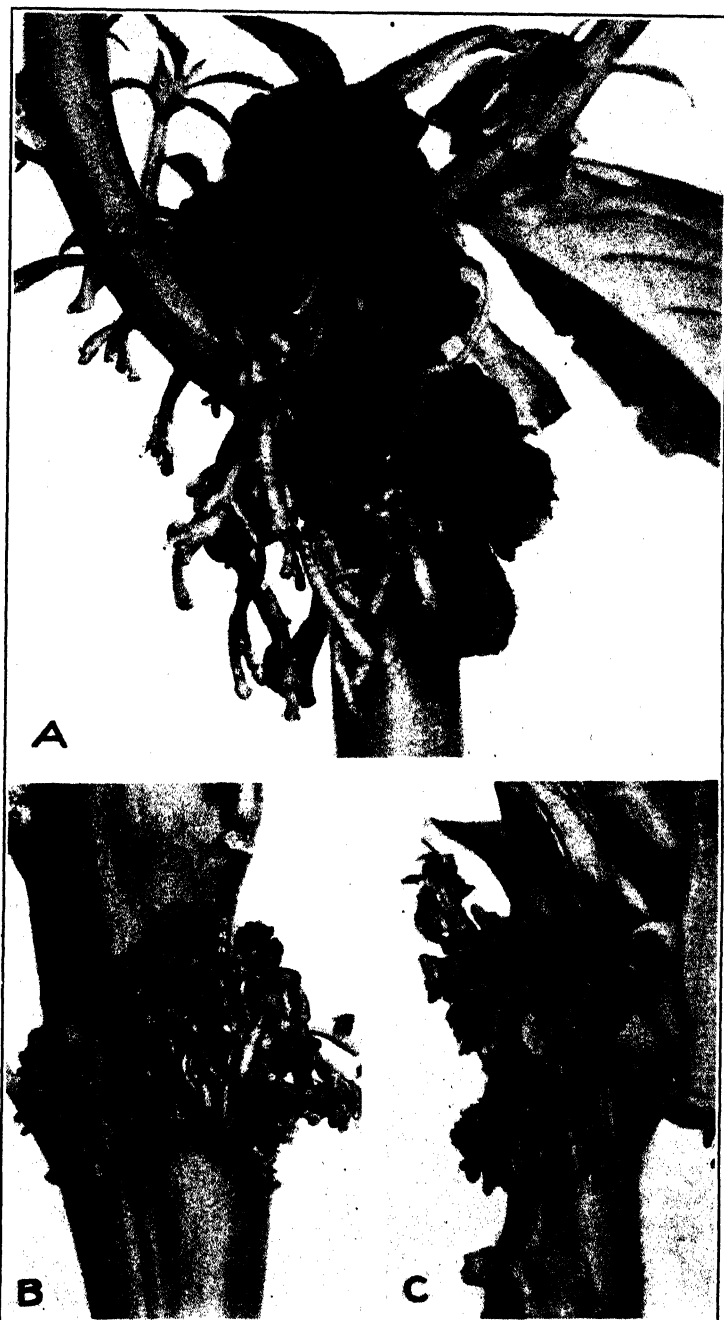


FIGURE 10.—*Impatiens balsamina* inoculated with different strains of *Bacterium tumefaciens*, June 15, 1928, and photographed September 17, 1928: A, Poplar strain; B, apple hairy-root strain; C, reisolated strain from apple hairy root. All natural size

STIMULATION OF ROOT PRIMORDIA TO ROOT FORMATION

Neither the rose nor the bean stems inoculated with the isolation from the apple hairy root produced roots in conjunction with the tumors (figs. 2, Ba, Bb, and C), but some of the tumor surfaces were roughened somewhat by root primordia. The bean tumors were stimulated to root production by chilling the whole plant in the refrigerator at a temperature of 10° to 14° C. for 20 hours, and then exposing it to a temperature of 25° to 27°. The primordia would extend into little roots 2 to 3 mm. long in four to eight hours. Some of the primordia that did not develop into roots in 24 hours could be made to do so by several successive changes from cold to warm temperatures. The change from cold to warm temperatures seemed to be necessary, as a pot of bean plants left in the refrigerator for 5 successive days did not show any root extensions from the three tumors until it had been in a warm room for about six hours. The tumors with smooth surfaces did not respond to the treatment, for roots did not appear.

The rose tumors with root primordia were stimulated to produce roots by being covered with sphagnum moss which was kept moist. Three of the eight tumors covered had produced roots when the sphagnum was removed after seven days. The stimulus here may have been a change of temperature also, for in a hot greenhouse evaporation must take place from the moist sphagnum; this could reduce the temperature of the tumor and might cause a stimulation to root development.

The roots induced by the ice-box and moss-ball stimulation were from 2 to 8 mm. in length. In some cases they were longer, but the tips dried back in the warm room as the root extended and true measurements could not be made. The roots of the rose tumors may have formed in a short time, but a week had elapsed when the moss ball was removed. One root from a rose tumor was 15 mm. long when uncovered, but the root was too fragile to survive the period required for photographing.

DISCUSSION

Can this tendency to root production of the crown-gall organism be an indication of a primitive soil form and the first manifestation of its pathogenicity? And, further, can it be that after passing through the apple or some other host and back into the soil the organism shows more active pathogenic features? And if so, why could not these more active features be manifested in the more definite tumor form instead of roots? This first act in pathogenicity might explain to some extent the slow adaptation of *Bacterium tumefaciens* to the apple as a host and the more frequent hairy-root type of crown gall which has been so confusing to nurserymen, and it might also explain the real tumor form of crown gall on apple to be produced by an organism beyond the first adaptation stage to the apple as host.

The organism reisolated from apple hairy root on daisy (fig. 4, Aa) produces roots and tumors more rapidly than the original isolation, and it grows more rapidly and profusely in culture media than does its forbear. That the crown-gall organism in general is an adaptive one is shown by the many plants that it infects, for with the exception of the monocotyledons, outgrowths caused by it have been found on most of our common fruits, vegetables, and ornamental plants.

The fasciation of stems of various plants like the sweet pea and the nasturtium known to be caused by the crown-gall organism might be produced by the same soil pathogenic form demonstrating its pathogenicity at the crown of a plant by fasciation instead of root or tumor production. The writer found that the organism isolated from fasciated sweet-pea stems was a weakly pathogenic one which would infect tiny sweet-pea seedlings but not plants 5 or more inches tall. The percentage of infection in the seedlings was only 37.

It is not known why some isolations of the apple strain are weakly parasitic and others actively parasitic. It may be that the less active organism is the primitive crown-gall parasite, or that it has had to survive a long residence in the soil between the times of host residence, while the more active one has passed through and infected one or more apple trees in succession and so increased its virulence. Or it may be that those very active crown-gall strains such as peach, hop, and daisy have had still more passages through various hosts, each time becoming more adaptable and virulent before returning to the soil.

SUMMARY

The crown-gall organism (*Bacterium tumefaciens*) can stimulate plants to produce roots as well as tumors. The strain isolated from apple trees produces both roots and tumors. Both the hairy-root and the tumor types of infection produced by the apple strain are considered symptoms or manifestations of the same disease.

The apple strain is slower to infect susceptible hosts than other strains except the rose. It is also slow to infect the apple tree.

The Paris daisy and *Impatiens balsamina* respond to inoculations with the apple strain by producing masses of roots and small tumors; the rose and bean stems by producing definite tumors instead of masses of roots; *Bryophyllum pinnatum* by producing masses of roots and tumors without roots. Tobacco, geranium, and Ricinus are very indifferent hosts on which a few inconspicuous outgrowths without roots are produced in two to four months. The results on tomato were negative.

When first isolated the apple strain differs somewhat in appearance from the other crown-gall strains (the rose excepted) when grown in culture media. These differences disappear when the apple strain is passed through another host and reisolated. These reisolation colonies tend to produce infection in less time than the original colonies.

Like the other strains of *Bacterium tumefaciens*, the apple strain is an adaptable one (though less so than the other strains), and when it passes through another host than the apple it becomes more virulent.

Root production is not limited to the apple strain of *Bacterium tumefaciens*, for the peach, hop, poplar, chrysanthemum, and other strains also produce roots.

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THE DISTRIBUTION OF VITAMIN A IN SOME CORN-MILLING PRODUCTS¹

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INTRODUCTION

It was shown by Coward in 1923 (1)³ that when whole yellow corn is used as the source of fat-soluble vitamins, large quantities are necessary to produce growth in rats which have been depleted of these vitamins. In more recent investigations Steenbock and Coward, (2, 21) have demonstrated that a much smaller quantity of whole yellow corn supplies sufficient vitamin A to produce a healthy condition in test animals if vitamin D is furnished in the basal ration. These results indicate, as suggested by the investigators, (1) that yellow corn is a remarkably poor source of vitamin D and that previous failure to promote growth on a low level of whole yellow corn was due to a deficiency of vitamin D rather than to a deficiency of vitamin A, and (2) that whole yellow corn is a rich source of vitamin A.

Millhouse and Croll,⁴ using the hand-dissected structures of whole yellow corn, found that the fat-soluble vitamins of yellow maize are located in the pigmented endosperm of the kernel. Steenbock and Coward (2, 21), also using hand-dissected corn, could state with greater certainty that vitamin A is concentrated in the endosperm of the yellow corn kernel, since in their study an irradiated basal diet furnished the antirachitic factor.

In the work here reported a qualitative and quantitative study of the distribution of vitamin A was made on all of the by-products recovered from the wet-milling process for the commercial separation of cornstarch from whole yellow corn.

At the time the milling products included in this study were obtained, yellow corn only was used by the milling company from which they were secured.⁵ The chemist reported a recovery of the whole yellow corn from the wet-milling process as follows:

Milling product	Per cent
Starch.....	65.0
Corn germs.....	7.0
Corn-germ meal.....	3.5
Crude corn oil.....	3.4
Grits.....	3.6
Gluten.....	10.0
Steep water (dried).....	5.0
Bran (reel slop).....	6.8
Gluten feed.....	25.4

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² Grateful acknowledgment is made to Hilda Croll Koser, under whose direction this study was begun.

³ Reference is made by number (italic) to "Literature cited," p. 779.

⁴ MILLHOUSE, L. E., and CROLL, H. Unpublished data.

⁵ Thanks are extended to the Staley Corn Products Co., Decatur, Ill., for the milling products used in this study.

The wet-milling process is a well-known commercial procedure for the preparation of cornstarch. The process as described by Rolfe (13) does not differ except in unimportant and minor details

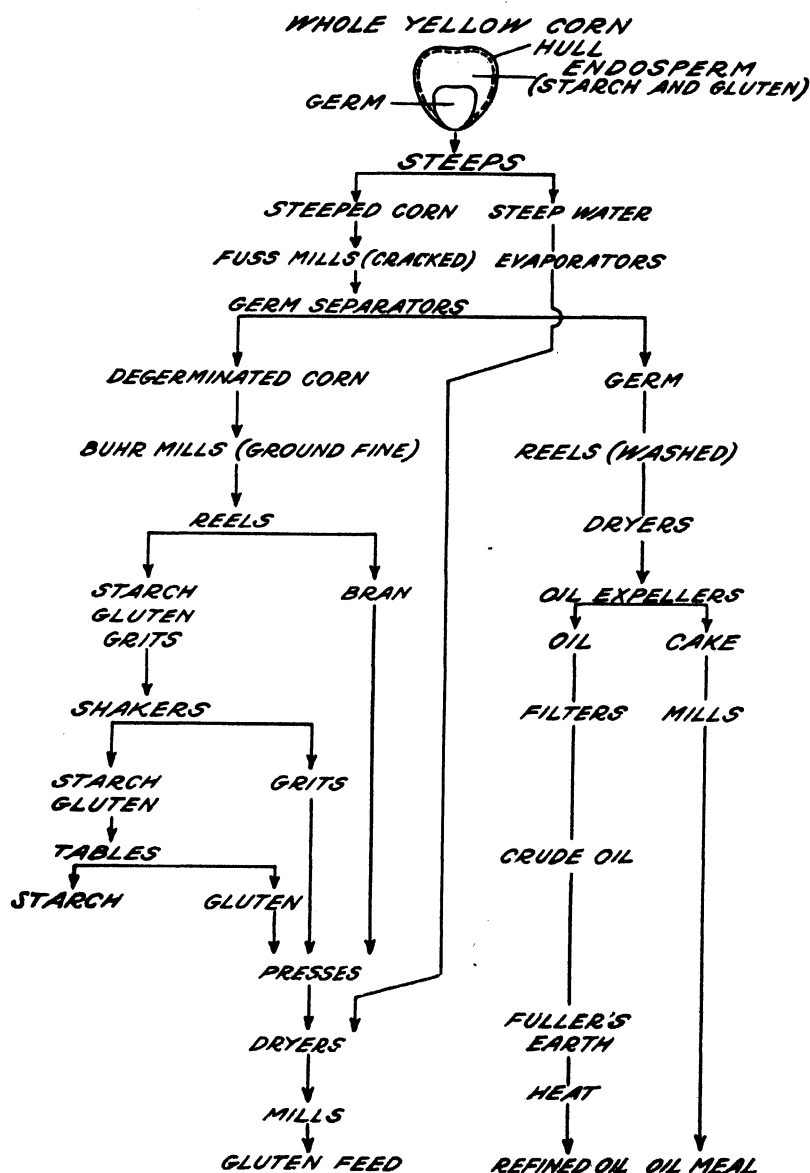


FIGURE 1.—Flow sheet indicating the relation of various corn-milling products to the structure of the corn kernel

from that employed by the milling company from which the products used in this work were obtained. It is almost entirely a physical rather than a chemical procedure involving comparatively

low temperatures and little chance for oxidation. The flow sheet in Figure 1 is a condensed diagram indicating the relation of the products here tested to the structure of the corn kernel and the general method of their separation by the wet-milling process.

EXPERIMENTAL PROCEDURE

Ophthalmia was developed in approximately 125 rats of the albino variety bred in the writers' own colony. Three series of animals were used in this study. In order to obtain rats of standard depletion in vitamin A, as Smith and Chick (19), Sherman and Storms (18), and other workers have found necessary, the mothers were given a diet low in this vitamin. For several weeks prior to and during breeding, and throughout the lactation period, a diet, essentially that used by Sherman (15, 16, 18), was fed. The diet consisted of two-thirds ground whole wheat and one-third whole-milk powder, with the addition of sodium chloride in the proportion of 1 per cent and meat scrap in the proportion of 5 per cent of the total weight of the wheat and

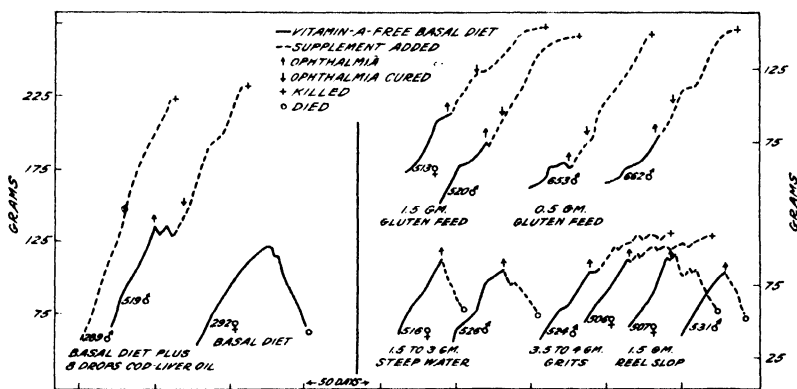


FIGURE 2.—Weight curves of rats receiving vitamin-A-free diets supplemented by various corn-milling products. When the vitamin-A-free basal diet was supplemented by 1.5 or 0.5 gm. of gluten feed daily, ophthalmia was cured; when the supplement was grits, ophthalmia was not cured and growth was very slow; when steep water or reel slop was used, ophthalmia was not cured and death resulted

milk powder. On the second or third day after birth the litters were reduced to eight young each. The experimental period was started when the young were 28 to 30 days of age.

The usual methods for individual caging, feeding, and caring for experimental animals were employed. Raised-bottom cages were used. The rats were weighed once a week for the first three or four weeks and then two or three times a week after the development of ophthalmia and during the succeeding test period. The food intake was carefully noted at the time the animal was weighed and whenever a new supply of food was given. In order to reduce the error incurred by the scattering of food, 500 c. c. porcelain jars with screw tops, having an opening about 1½ inches in diameter, were used. The jars were filled about one-third full of food.

An attempt was made to determine the standard rate of growth for recovery of animals in series 2 and 3 by feeding cod-liver oil, 8 drops daily, after the appearance of ophthalmia. (Table 1 and fig. 2.) This is probably a better method by which to judge the extent of the

vitamin deficiency than the uninterrupted growth of an animal fed cod-liver oil from the beginning of the experimental period (9). (Table 2.) A "negative control" animal was maintained in each litter on the vitamin-A-free basal diet (Sherman, 17). (Table 3.)

TABLE 1.—*The effect of cod-liver oil on ophthalmia and growth of vitamin-A-deficient rats*

[Positive controls]

No. and sex of rat	Litter No.	Initial age	Initial weight	Time of ophthalmia incidence and weight of animal		Time of recovery from ophthalmia and weight of animal		Amount of supplement daily	Average weekly food intake (vitamin-A-free basal diet)		Duration of test period ^a	Final age	Final weight
				Days after experiment began	Body weight	Days after addition of supplement to diet	Body weight		Before addition of supplement	After addition of supplement			
		Days	Gm.		Gm.		Gms.	Drops	Gm.	Gm.	Weeks	Days	Gm.
519♂	6	31	56	27	128	7	142	8	61.0	74.0	6	114	231
645♀	10	24	40	34	45	11	82	8	19.0	41.0	7	119	157
671♂	13	30	34	28	71	9	119	8	38.6	69.0	5	111	165
658♀	12	29	44	31	78	9	119	8	42.2	63.0	5	111	162
817♀	17	29	55	30	158	6	176	8	65.5	83.0	3	79	205
821♂	17	29	64	34	164	7	186	8	68.2	68.5	4	79	220
832♂	14	29	53	38	168	10	206	8	65.6	67.7	4	83	228
839♀	15	29	46	28	120	12	158	8	55.5	50.6	4	83	179

^a The animals were all killed at the end of the test period.

TABLE 2.—*The prevention of ophthalmia and the growth of rats fed a vitamin-A-free basal diet supplemented by cod-liver oil*

[Positive controls]

No. and sex of rat	Litter No.	Initial age and weight		Amount of supplement fed daily	Average weekly food intake (vitamin-A free basal diet)	Duration of test period ^a	Final age	Final weight
		Days	Gm.					
305♀	3	29	46	8	58.0	8	85	163
297♂	2	29	44	8	47.4	12	113	188
289♂	1	28	43	8	62.0	8	84	210
535♂	8	26	40	8	59.0	7	74	170
511♀	5	32	46	8	51.2	7	81	144
655♀	11	29	36	8	45.9	10½	103	158
672♂	13	30	23	8	56.4	10	101	165
840♀	15	29	39	8	60.1	8	83	165

^a The animals were all killed at the end of the test period.

TABLE 3.—*The effect of vitamin-A-free basal diet only on ophthalmia and growth of vitamin-A-deficient rats*

[Negative controls]

No. and sex of rat	Litter No.	Initial age	Initial weight	Time of ophthalmia incidence and weight of animal		Time of death from ophthalmia and weight of animal		Average weekly food (vitamin-A-free basal diet)		Duration of test period after incidence of ophthalmia *	Final age
				Days after experiment began	Body weight	Days after development of ophthalmia	Weight at death	Before development of ophthalmia	After development of ophthalmia		
		Days	Gm.		Gm.		Gm.	Gm.	Gm.	Weeks	Days
308♂	3	29	45	35	100	8	89	29.2	26.0	1	72
208♂	2	29	48	35	100	47	62	36.0	31.0	7	111
292♀	1	28	42	28	106	45	70	48.0	35.6	6	101
322♀	4	29	40	37	120	42	70	55.4	28.0	6	108
518♀	6	31	48	27	101	23	78	54.2	44.0	3	81
523♀	7	26	36	29	73	21	52	38.7	28.0	3	76
504♂	5	32	50	34	88	7	100	42.0	41.0	1	73
534♂	8	26	42	27	86	14	80	48.0	32.0	2	67
641♀	10	24	36	34	62	50	45	40.0	39.4	7	108
640♀	9	31	52	34	52	53	62	17.8	26.0	7	118
649♀	11	29	36	33	60	33	60	33.3	47.2	4	95
657♂	12	29	44	33	68	33	76	31.4	45.6	4	95
665♀	13	29	46	28	68	38	63	32.5	31.0	5	95
844♀	16	28	40	28	121	28	104	63.0	54.0	4	84
820♂	17	29	62	14	118	26	153	75.0	71.0	4	71
852♂	19	30	58	28	158	28	123	80.0	49.5	4	86
836♂	15	29	42	24	120	16	120	52.0	62.0	2	69
828♀	14	29	47	27	125	13	130	50.0	70.0	2	69

* The test period terminated with the death of the animal in each case.

A vitamin-A-free basal food was given the test animals, which they were allowed to eat ad libitum. The antirachitic factor was supplied in the food by irradiation in shallow agate pans at a distance of 20 inches from the lamp for one-half hour. The food was stirred at the end of each 15 minutes of irradiation (21, 20). A Hanovia luxor mercury arc lamp was used.

The basal diet was composed of the ingredients in the percentage amounts given below:

	Per cent
Extracted casein.....	18
Osborne-Mendel salt mixture.....	4
Crieso ⁶	20
Starch.....	57
Sodium chloride.....	1

The casein was purified by the alcohol-extraction method of Osborne and Mendel (10, 11). It was then air-dried for several days and subsequently spread out in shallow pans and heated from 8 to 12 hours in gas ovens at 120° C. (Drummond (3, 6, 7).)

The basal food was mixed and stored in glass jars in a refrigerator. If it was to be used within a short time it was first irradiated and then stored in the refrigerator. A week's supply was usually irradiated at one time.

The water-soluble vitamin-B factors were supplied by dried brewers' yeast, five-tenths gram per animal daily, fed separately.

The development of definite ophthalmia was taken as a sign of vitamin-A deficiency. (Morgan (9), Steenbock and Coward (21),

⁶ Drummond (5), Daniels and Laughlin (4).

Steenbock, Sell, and Buell (22).) With the depleted animals used, definite eye symptoms almost invariably preceded cessation of growth and developed from 25 to 35 days after the experimental period began.

As soon as the eye symptoms became persistent, the test food was added to the diet. It was given separately in definitely weighed portions, or later, especially in the case of foods not entirely eaten when fed separately, as a definite part of the basal diet. The supplement replaced the same percentage of starch, or Crisco, or parts of both the starch and the Crisco; when thus mixed with the basal food, the test material was added after the irradiation of the purified ingredients. (Willimott and Wokee (23).) When fed separately the daily portion of the test food was moistened with distilled water immediately before feeding, for all of the foods, with the exception of the oils, were extremely dry and powdery, and the moistening reduced possible loss by air drafts.

The vitamin-A value of the test food was judged chiefly by the minimal dosage necessary for the ultimate cure of the eyes. (Steenbock and Coward (21).) A resumption of body growth was also observed.

EXPERIMENTAL RESULTS

In order to make this investigation quantitative in nature, it was necessary first to conduct a preliminary qualitative study of the corn-milling products. Each of the products was fed in accurately weighed amounts of 1.5 gm. daily to at least two rats in which ophthalmia had developed. The results obtained in this first series of animals as indicated by the ultimate positive or negative cure of ophthalmia were without exception as shown in Table 4.

TABLE 4.—The effect on ophthalmia of various corn-milling products when used as supplements to a vitamin-A-free diet

Supplement	Amount fed daily per animal	Number of rats	Effect on ophthalmia	Effect on growth
	<i>Grams</i>			
Steep water.....	1.5	3	Not cured.....	Decline and death.
	2.3	1do.....	Do.
	3.0	1do.....	Do.
Reel slop.....	1.5	3do.....	Do.
	1.8	1do.....	Do.
	2.3	1do.....	Do.
	1.5	2do.....	
Grits.....	2.6	1do.....	Very slow decline; all but 1 lived.
	3.4	1do.....	
	4.0	1do.....	
	1.5	2	Rapid cure.....	Normal growth.
Gluten.....	1.0	5do.....	Do.
	.5	2do.....	Do.
	.25	3do.....	Do.
	.1	3	Not cured.....	Growth finally arrested; 1 died.
Gluten feed.....	1.5	8	Rapid cure.....	Normal growth.
	1.5	3	Not cured.....	Slowly declined; 1 died.
Germ.....	2.0	4do.....	All 4 died.
	1.5	2do.....	Rapid decline; death.
Germ meal.....	3.0	2do.....	Do.
	1.5 to 1.7	5	Slow cure.....	Lived and grew.
Crude corn oil.....	.9 to 1.2	6do.....	Do.
	.28 to .4	3	Not cured.....	All died.
	1.5	2do.....	Decline and death.
Refined corn oil.....	1.0	1do.....	Do.
	.6	1do.....	Do.
	1.5	5	Rapid cure.....	Normal growth.
	1.0	8	More slowly cured..	Do.
Whole yellow corn..	.75	5	Never permanently cured.	Growth approaching normal.
	.5	5do.....	Growth arrested; 3 died.
	.25	5	Not cured.....	Low rate of growth; all died.

In the case of steep water, reel slop, and grits, which were more or less poorly consumed as separate fractions, an attempt was made to feed at a higher level in order to test for traces of the vitamin. These three by-products were fed, therefore, as 50 per cent of the diet, replacing the same percentage of starch in the basal ration. As a result, the daily dosage was increased as shown in Table 4. In each case, as the table shows, despite the higher level reached in the feeding of the supplement, there was neither a cure of ophthalmia nor a resumption of growth. The animals fed the steep water exhibited extremely sore eyes, with considerable pus and hemorrhage, and in some instances complete atrophy of the eyeball. The feces were watery and had the characteristic brown color of the steep water. The animals receiving the reel slop or bran supplement developed extreme ophthalmia to the same extent, apparently, as those on the steep water. The feces, however, were very large, due, probably, to the great extent of indigestible crude fiber in the reel slop. The grits supplement was better consumed than the steep water or the reel slop. There was a slow development of extreme ophthalmia in the case of animals on this fraction, but at no time were the eyes cured. It is apparent from these results, that the three by-products named above are almost if not totally lacking in vitamin A. (Fig. 2.)

Gluten feed in 1.5-gm. daily amounts produced a rapid cure of ophthalmia in eight rats. (Table 4.) The gluten feed is composed of reel slop, grits, steep water, and gluten. (Fig. 1.) Since all these products except gluten when tested separately were almost if not totally lacking in vitamin A, it seemed necessary only to test the gluten in a quantitative manner for this vitamin. The various levels of feeding used and the results obtained are shown in Table 5.

TABLE 5.—The effect of feeding corn gluten on ophthalmia and growth of vitamin-A-deficient rats

No. and sex of rat	Litter No.	Initial age	Initial weight	Time of ophthalmia incidence and weight of animal		Time of recovery from ophthalmia and weight of animal		Ophthalmia not cured		Amount of supplement fed daily	Average weekly food intake (vitamin-A-free basal diet)		Duration of test period*	Final age	Final weight
				Days after experiment began	Body weight	Days after addition of supplement to diet	Body weight	Days after addition of supplement to diet	Body weight		Before addition of supplement	After addition of supplement			
<i>Days</i>		<i>Gm.</i>		<i>Gm.</i>		<i>Gm.</i>		<i>Gm.</i>		<i>Gm.</i>	<i>Gm.</i>	<i>Weeks</i>	<i>Days</i>		<i>Gm.</i>
306♂	3	29	46	39	98	10	110			1.50	41.0	44.8	8	120	170
294♀	1	28	43	28	113	14	144			1.50	35.0	54.3	7	112	176
521♂	7	26	31	29	70	10	93			1.00	45.5	44.8	8	109	138
529♂	8	26	42	29	89	10	102			1.00	56.5	55.5	8	109	182
533♀	8	26	38	27	74	10	84			1.00	39.2	48.4	8	109	152
508♀	5	32	50	32	106	6	112			1.00	58.0	59.0	7	115	168
515♂	6	31	50	32	114	6	123			1.00	53.0	60.0	7	114	195
504♂	5	32	36	34	88	14	122			1.00	42.0	50.0	6	115	172
653♂	11	29	44	29	54	6	71			.50	33.5	44.1	8	117	160
663♂	12	29	46	33	75	14	108			.50	31.4	44.7	8	117	175
650♂	11	29	40	32	61	14	77			.25	29.3	37.1	8	117	136
660♀	12	29	44	33	83	9	102			.25	27.0	42.0	8	117	147
666♀	13	29	42	32	66	14	85			.25	36.8	40.0	8	117	122
661♂	12	29	43	33	72			34	95	.10	36.6	37.6	5	96	95
667♀	13	29	50	33	78			55	121	.10	43.1	37.2	8	117	121
651♂	11	29	37	33	58			55	93	.10	37.7	38.0	8	117	93

* All animals were killed except 661 which died with ophthalmia.

As previously stated, gluten is recovered in quantity equivalent to about 10 per cent of the whole corn. If then all the vitamin A of the whole yellow corn were concentrated in the gluten, similar results should be obtained with 0.1 gm. of gluten and 1 gm. of whole yellow corn fed daily. One gram of the whole yellow corn fed daily gave a comparatively rapid cure of ophthalmia and produced normal growth. It was found, however, that 0.1 gm. of gluten was not sufficient to cure or even to arrest ophthalmia. (Fig. 3 and Table 5.) One-fourth of a gram of gluten, however, cured the eye lesions, and, in addition, produced growth in the vitamin-A-depleted rats. Some of the vitamin, therefore, must have been lost in the separation of gluten from the corn or is present in other structures of the corn kernel.

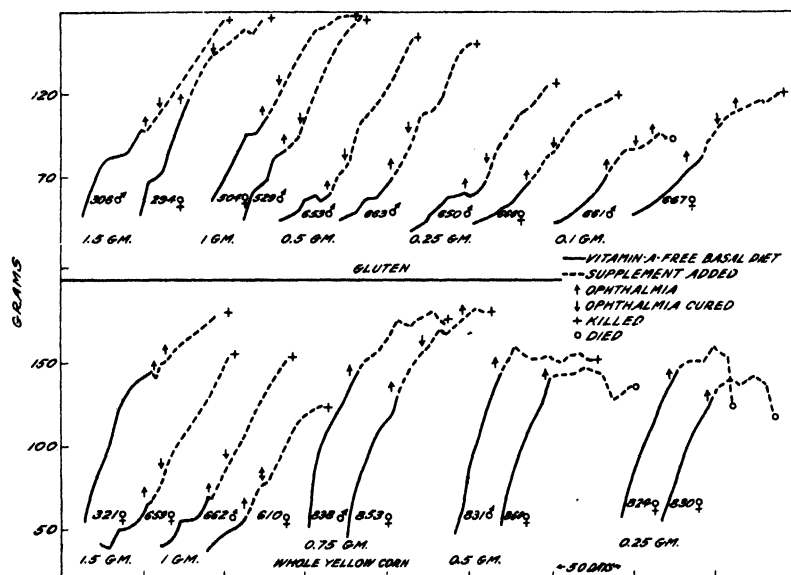


FIGURE 3.—Weight curves of rats receiving vitamin-A-free diets supplemented by various quantities of gluten and whole yellow corn

The results of the preliminary study indicated that some of the vitamin A of corn occurs in the germ structure of the kernel. A cure of ophthalmia was demonstrated with the crude corn oil. The germ itself, however, and the germ meal at the levels fed did not cure the eyes. The animals barely maintained weight and eventually died. The animals given the germ fared better than those given the germ meal. (Fig. 4.) But since a daily intake of 2 gm. of the germ was followed at best by only a slight improvement in the eye condition (Table 4), it was concluded that the small amount of vitamin A present in the germ had to be pressed out in the germ oil before its presence could be detected. The results of the quantitative study of crude corn oil, which are summarized in Table 6, show that crude corn oil is comparatively rich in vitamin A. (Fig. 4.)

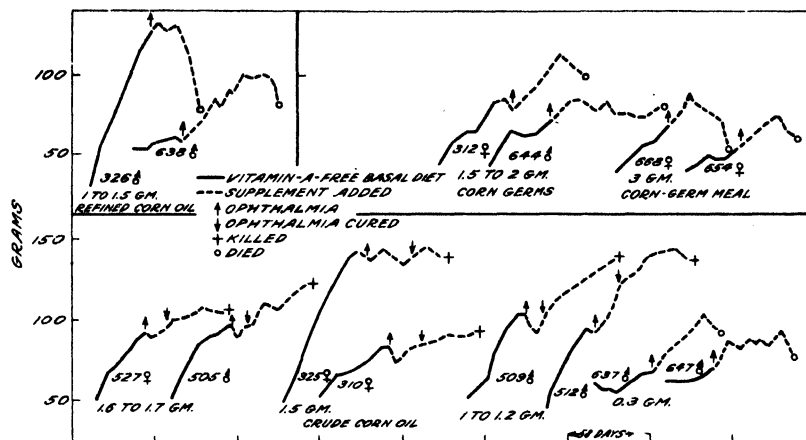


FIGURE 4.—Weight curves of rats receiving vitamin-A-free diets supplemented with various corn-milling products. When the vitamin-A-free basal diet was supplemented with one or more grams daily of crude corn oil, ophthalmia was cured and the animals lived; when the supplement was corn germs, corn-germ meal, or refined corn oil, ophthalmia was not cured and death resulted.

TABLE 6.—The effect of crude corn oil on ophthalmia and growth of vitamin-A-deficient rats

No. and sex of rat	Litter No.	Initial age	Time of ophthalmia incidence and weight of animal		Time of recovery from ophthalmia and weight of animal		Ophthalmia not cured		Amount of supplement fed daily	Average weekly food intake (vitamin-A-free basal diet)		Duration of test periods *	Final age	Final weight	
			Initial weight	Days after experiment began	Body weight	Days after addition of supplement	Body weight	Days after addition of supplement to diet		Body weight	Before addition of supplement				After addition of supplement
<i>Days</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Weeks</i>	<i>Days</i>	<i>Gm.</i>		
310♂	3	29	43	38	80	22	82			1.5	35.0	17.5	8	120	90
325♂	4	29	49	48	138	28	140			1.5	46.0	31.0	6	119	140
514♂	6	31	52	29	92	12	98			1.55	45.5	16.0	8	114	124
505♂	5	32	47	34	98	14	96			1.66	45.0	17.0	8	120	124
527♀	7	26	34	29	84	12	92			1.7	48.0	19.0	7	109	106
525♀	7	26	36	32	82	9	100			1.0	41.0	31.0	7	109	106
509♂	5	32	53	29	105	12	100			1.0	51.0	40.0	8	115	138
512♂	6	31	50	29	88	12	122			1.2	52.0	44.4	8	114	136
642♂	10	24	57	34	58	27	93			1.0	34.4	28.0	9	120	132
634♂	9	31	45	38	47	40	102			.9	23.6	26.7	8	127	124
643♂	10	24	33	34	54	30	86			1.0	35.0	34.4	9	120	118
646♀	10	24	35	34	58			46	74	.4	36.6	25.2	6½	104	74
637♀	9	31	55	38	64			52	87	.28	23.0	17.5	7½	111	87
647♂	10	24	35	34	38			51	57	.3	14.0	19.2	7½	109	57

^a All animals were killed except 646, 637, and 647, which died with ophthalmia.

Refined corn oil was not only incorporated in the basal diet to replace the 20 per cent Crisco, but was also fed separately in daily fractions. From the results shown in Table 4 and Figure 4 it is evident that there is little if any vitamin A in refined corn oil. It may be concluded that there is sufficient heat under oxidative conditions involved in the refining process to destroy all of the vitamin A that may have been present in the crude corn oil.

A quantitative study of the unmilled yellow corn was undertaken. The results obtained (Table 7) show that 1 gm. daily, or approximately

11 per cent, is the minimum requirement for permanent cure of ophthalmia and normal growth. Temporary cure of the eyes was obtained with 0.75 gm. daily, or approximately 9 per cent, and even with as little as 0.5 gm. daily, but the eyes again became extremely sore, a general decline occurred, and death resulted in most cases.

TABLE 7.—*The effect of (ground) whole yellow corn on ophthalmia and growth of vitamin-A-deficient rats*

No. and sex of rat	Litter No.	Initial age	Initial weight	Time of ophthalmia incidence and weight of animal		Time of recovery from ophthalmia and weight of animal		Ophthalmia not cured		Amount of supplement fed daily	Average weekly food intake (vitamin-A-free basal diet)		Duration of test periods	Final age	Final weight
				Days after experiment began	Body weight	Days after addition of supplement to diet	Body weight	Days after addition of supplement to diet	Body weight		Before addition of supplement	After addition of supplement			
		Days	Gm.		Gm.		Gm.		Gm.	Gm.	Gm.	Gm.	Weeks	Days	Gm.
321♂	4	29	54	44	143	5	154	—	—	1.50	60.3	50.7	5.5	112	180
307♂	3	29	47	39	98	10	110	—	—	1.50	39.1	46.0	7.5	120	176
304♀	2	29	50	35	90	11	95	—	—	1.50	33.2	40.4	8	120	178
659♂	12	29	42	33	68	6	79	—	—	1.50	28.8	39.0	8	117	153
652♀	11	29	48	33	66	9	82	—	—	1.50	25.6	43.0	8	117	155
656♀	11	29	44	33	62	7	89	—	—	1.0	27.6	47.0	8	117	178
662♂	11	29	42	41	70	7	91	—	—	1.0	27.0	43.7	8	117	167
664♂	12	29	42	31	74	11	96	—	—	1.0	29.2	47.5	8	117	162
669♀	13	29	36	33	68	7	76	—	—	1.0	26.2	43.3	8	117	148
670♀	13	30	32	30	58	9	78	—	—	1.0	46.7	45.4	8	117	135
829♀	14	29	49	28	133	12	147	—	—	1.0	66.0	71.5	8	113	170
837♀	15	29	44	28	123	12	132	—	—	1.0	63.5	74.6	8	113	167
845♀	16	28	51	28	133	18	147	—	—	1.0	59.2	66.7	8	110	178
822♂	17	29	63	30	170	—	—	56	198	.75	78.0	48.0	8	115	198
838♂	15	29	48	28	140	—	—	56	174	.75	67.7	49.1	8	113	174
846♀	18	28	39	28	114	—	—	46	109	.75	58.0	44.3	7	102	109
853♀	19	30	51	28	136	—	—	54	181	.75	70.5	72.0	8	112	181
856♀	19	30	53	28	134	—	—	54	167	.75	60.0	52.0	8	112	167
823♀	17	29	60	30	149	—	—	56	168	.50	70.2	75.2	8	115	168
851♂	14	29	54	28	147	—	—	56	147	.50	63.2	44.6	8	113	147
847♀	18	28	57	34	156	—	—	41	153	.50	52.4	57.1	6	115	153
854♀	19	30	61	32	157	—	—	55	158	.50	50.1	50.0	8	117	158
860♀	20	30	51	32	145	—	—	48	138	.50	60.0	49.0	7	110	138
824♀	17	29	58	30	142	—	—	37	128	.25	70.5	74.4	5	96	128
830♀	14	29	54	28	128	—	—	37	116	.25	57.0	61.4	5	94	116
848♀	18	28	40	36	127	—	—	20	103	.25	61.6	47.3	3	84	103
855♀	19	30	46	29	127	—	—	35	133	.25	59.8	67.4	5	94	133
861♂	20	30	56	34	141	—	—	29	140	.25	60.2	66.2	4	93	140

¹ Animals 846, 847, 854, 860, 824, 830, 848, 855, and 861 died; the rest were killed.

As a result of this study a flow sheet similar to that in Figure 1 may be drawn to indicate the distribution of vitamin A in the same milling products. Such a sheet is shown in Figure 5.

The condition of typical animals while on a diet deficient in vitamin A and after recovery from the effects of vitamin-A depletion is shown in Figure 6.

THE RELATION BETWEEN VITAMIN-A-CONTENT AND YELLOW PIGMENTATION

The high concentration of vitamin A in highly pigmented products of yellow corn, gluten, and crude corn oil as found in this study demonstrates the association of vitamin A with yellow pigmentation. Coward (1), in 1923, stated that some lipochrome, generally carotin, is always associated with the vitamin A in plant tissues and that where the carotin is found, particularly carotin exposed to sunlight,

there also the vitamin may be expected to be present. However, various other workers, as for example, Palmer and Kennedy (12) in 1921, Steenbock, Sell, and Buell (22) in 1921, and Drummond and Coward (7) and Rosenheim and Drummond (14) in 1920, have noted exceptions in the association of vitamin A with yellow pigmentation.

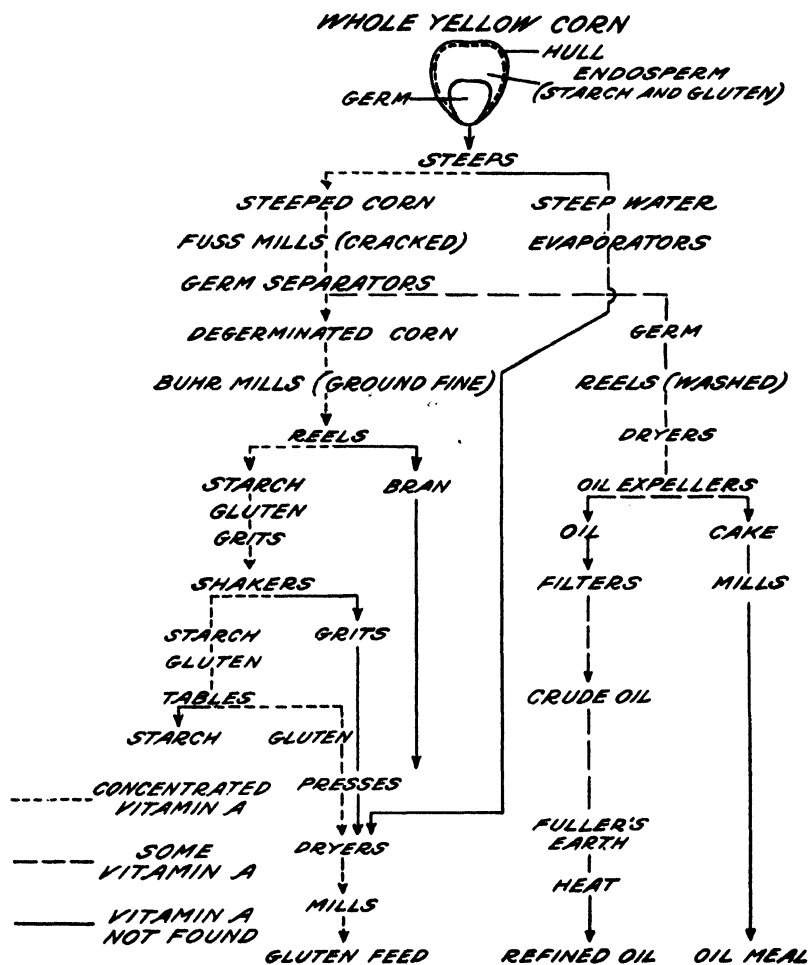


FIGURE 5.—Flow sheet indicating the distribution of vitamin A in various corn-milling products

Hauge and Trost (8), in a recent inheritance study of the association of vitamin A and yellow endosperm kernel, concluded that any genetic relationship existing between these two factors must be one of close linkage. They found that vitamin A was transmitted exclusively with yellow endosperm through the process of crossing and segregation.

SUMMARY

The rate of growth, and the recovery or nonrecovery from ophthalmia of vitamin-A-depleted rats were observed when their vitamin-A-deficient basal diets were supplemented with whole yellow corn and with various corn-milling products.

Whole yellow corn was found to be rich in vitamin A. Rapid growth was obtained when amounts of 1.5 gm. per animal were fed daily. The minimal amount needed to cure ophthalmia was 1 gm. daily, or approximately 11 per cent of the total food intake.

Gluten feed administered in amounts of 1.5 gm. daily rapidly cured the ophthalmic eyes of vitamin-A-depleted rats. Three of the four milling products that constitute the gluten feed—namely, steep water, reel slop, and grits—failed in every instance to effect a cure; gluten, the fourth constituent, was found to be extremely potent. The minimum amount of gluten required to cure ophthalmia was 0.25 gm. fed daily, and this amount also produced normal growth.

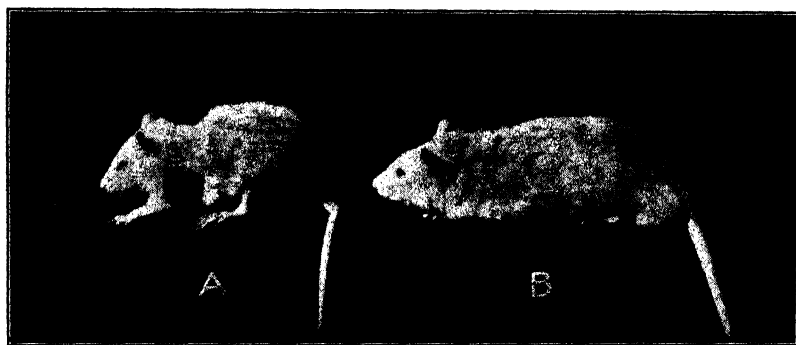


FIGURE 6.—Typical animals representing the vitamin-A-deficient rat, and the rat which has recovered from vitamin-A deficiency. A.—In the case of rat 531♂ ophthalmia was not arrested and a decline in growth resulted when the diet consisted of steep water, grits, reel slop, corn germs, corn-germ meal, and refined corn oil as sole sources of vitamin A. B.—When ophthalmic animals were fed yellow corn, gluten, gluten feed, or crude corn oil, the ophthalmia was cured and there was a resumption of growth, the animals finally appearing like rat 519♂

This finding shows that vitamin A is concentrated in the endosperm of the yellow-maize kernel. It furthermore unquestionably indicates that the vitamin-A concentration is greatest in that part of the endosperm lying next to the seed coats—the pigmented, nitrogenous outer layer of the endosperm.

Crude corn oil was found to cure ophthalmic rats when administered in daily doses of 1.5 gm. Corn germs, the source of the crude corn oil, were found to be so devoid of vitamin A that no cure could be obtained even when they were fed at a level of 2 gm. daily. The germ meal was found totally lacking in vitamin A. It was concluded that the small amount of vitamin A not found in the gluten is concentrated in or associated with the corn oil of the yellow corn germ and that it follows the oil in its removal from the germ. Refined corn oil was found to be totally lacking in antiophthalmic qualities and hence in vitamin A.

A high vitamin-A content was found associated with yellow pigmentation.

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THE INHERITANCE OF RHODE ISLAND RED CHICK DOWN-COLOR VARIATIONS AND THEIR RELATION TO COLOR VARIATIONS IN ADULT PLUMAGE¹

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INTRODUCTION

Any inheritance studies involving variations in the down of chicks have a special theoretical interest because they fall within the category of larval characteristics. Relatively few distinctly larval characters have been made the subject of genetic studies, yet the bearing of these characters upon the problem of the gene in its relation to development is a fundamental one. Color patterns of chick down may be considered as in the nature of larval characteristics for the reason that in many cases the pattern appears to bear little relation to that of the adult. It is true that each adult plumage pattern has a definite corresponding down pattern, but frequently the two patterns have little in common. Both adult and chick down patterns vary to a certain degree, but very little attention has been given to how variations of one affect the expression of the other.

In addition to its theoretical bearing this study has certain practical aspects since any information of predicative value to be obtained from the chicks regarding the color qualities of adults is of considerable value. Rhode Island Red chicks vary widely in the shade of the red color. The lightest individuals are cream colored with only a tinge of red while the darkest are a chocolate brown.

MATERIALS AND METHODS

The stock used in this study was the Single Comb Rhode Island Red flock of the Kansas Agricultural Experiment Station. The strain had been bred for several years as a production flock, and an effort has been made to maintain standard qualities. The average color of the flock is a good dark red, probably above the average quality of most production-bred flocks. As in most flocks of this breed, there is considerable individual variation in the shade of the red color.

In order to keep the classification of chick down color as uniform as possible throughout the study, a standard series of skins was prepared. The skins were arranged in a Riker specimen mount and given grade numbers ranging from 1 to 5. These grades of down color are shown in Plate 1. As soon as chicks were removed from the incubator they were wing-banded and graded according to the standard series. Lighting conditions were kept as uniform as possible while grading was being done.

For recording variations in adult color a detailed description was made at 6 months of age. Wherever possible, standards similar to

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the one used for down color were made up. For recording the shade of red of surface and under color of plumage a Riker mount standard was used. This was made by taking sections of plumage from individuals showing the existing range of color. The record was made by entering the number of the standard grade which most nearly matched the color of the individual. The eye color recorded was that of the iris. The variations noted were in the shade of red which was determined by comparison with a prepared color standard. The standard series used for adult surface and under color and for eye color are shown in Plate 2. Variations in the amount of black pigment in the primary and secondary wing feathers were

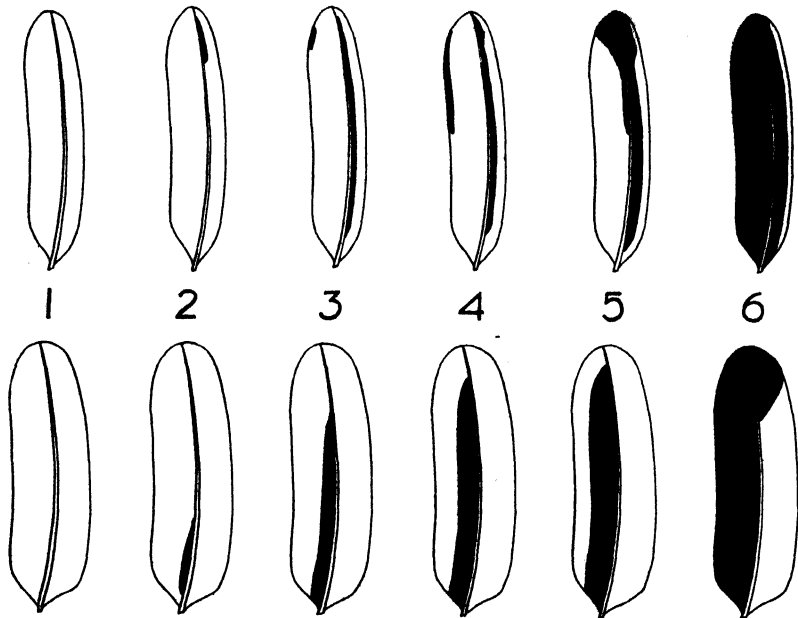
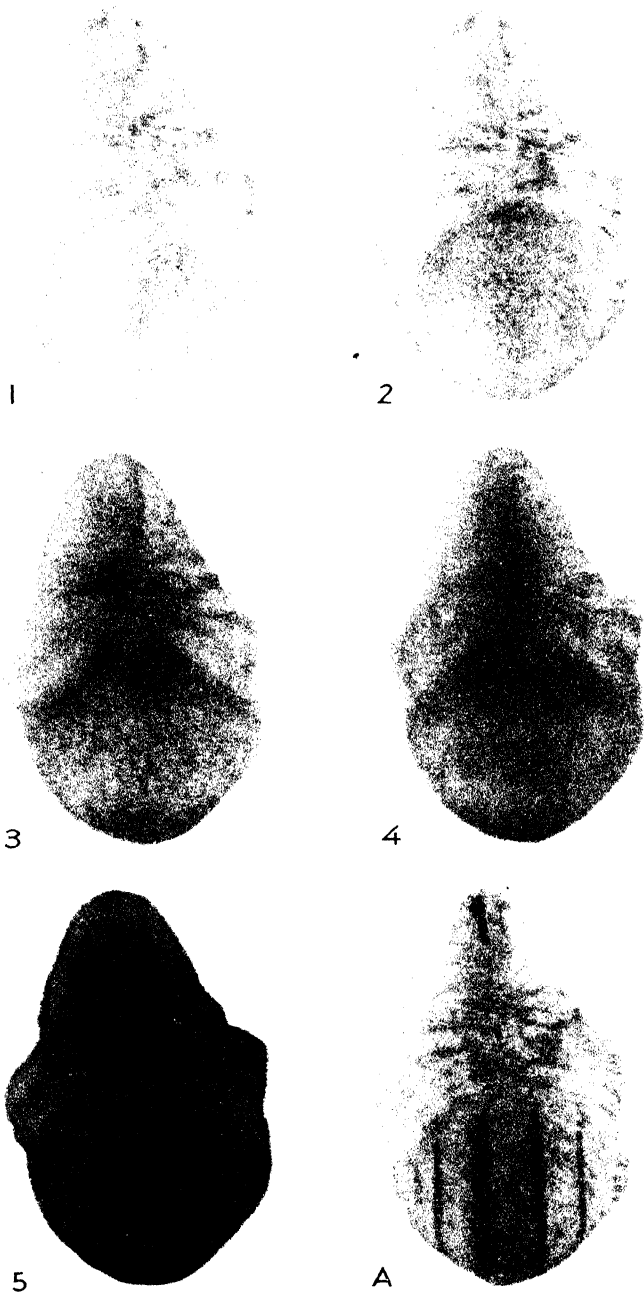


FIGURE 1.—Grade-number standards for variations in the amount of black pigment in the primary (upper series) and secondary (lower series) wing feathers of adult Rhode Island Red chickens

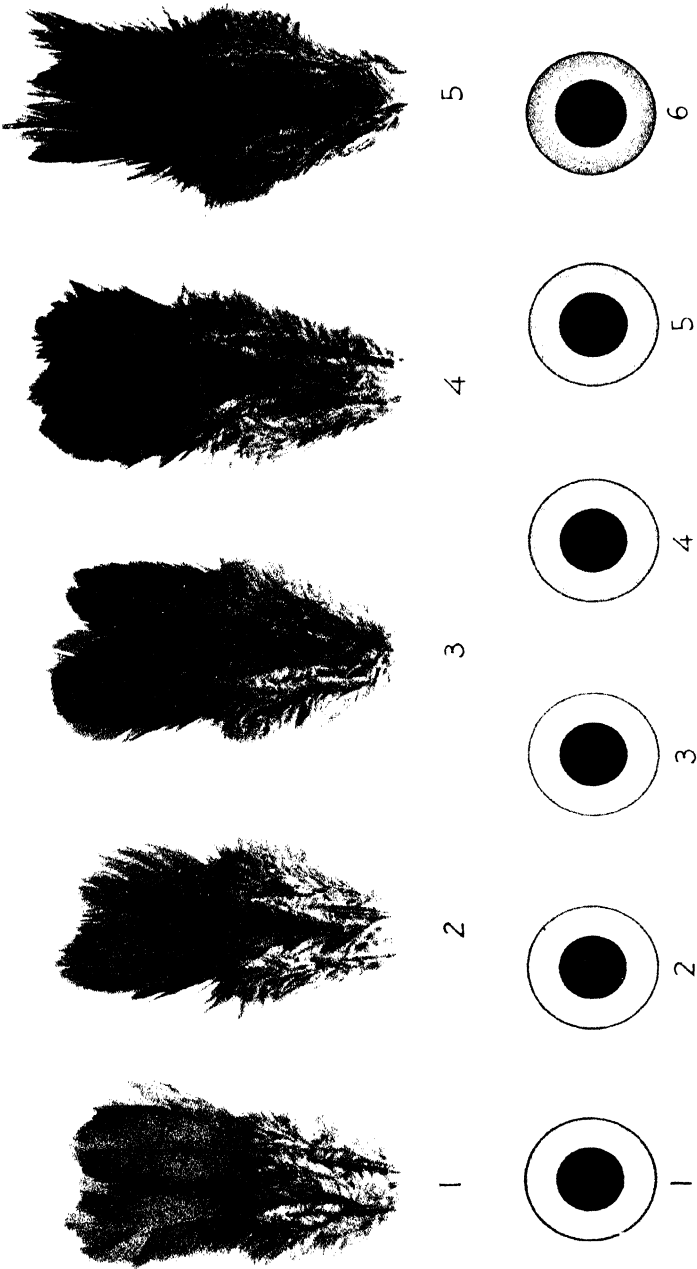
recorded by grade number given in a standard (fig. 1) showing the range of variations. The flight feathers used for recording the grade of black were the second full-length primary and the second secondary. In the description of the surface color the back, hackle, wing bow, and breast were graded separately. The occurrence of black pigment in any section of the surface was also recorded. In addition to recording the shade of under color, note was also made of the presence of smut² or of white in this portion of the plumage.

Because of the large number of birds involved it was impractical to preserve entire skins, but a fairly satisfactory substitute was devised. This was a feather card upon which sample feathers from seven representative sections of the plumage were mounted. The

² In the use of the term "smut" the writer has accepted the terminology of the poultry fancier. However, since this word has a different connotation in ordinary usage, the word "soot" would appear to describe much better the presence of black pigment in the under surface of the plumage.



Chicks 1 to 5 show the standard series used for grading down color. Chick A shows striping in an extreme degree



EASTMAN KODAK CO. BOSTON, U.S.A.

Standard color series used to grade adult surface and under color, and adult eye color. The tips of the feathers show the five grades of surface color. Feathers of the first four grades were taken from females but the grade 5 sample is from male plumage, since females seldom show the darkest color. Feathers 1 to 4 also show the four grades of under color.

regions represented were the saddle, hackle, wing bow, back, breast, and the primary and secondary wing feathers. For mounting the feathers a 8 by 5 inch card bearing a double row of loosely set wire staples was used. The feathers were slipped beneath the staples, after which the staples were clamped tightly by the use of a long-lipped pair of pliers. This made for each individual a permanent record, and these records were arranged by families in standard filing cases. Figure 2 shows a filled feather card.

The work upon inheritance of down color covers the period from 1924 to 1928, but the data upon relation of down to adult color are only for the breeding seasons of 1927 and 1928. Relationships of down and adult colors were recorded for the entire period, but only

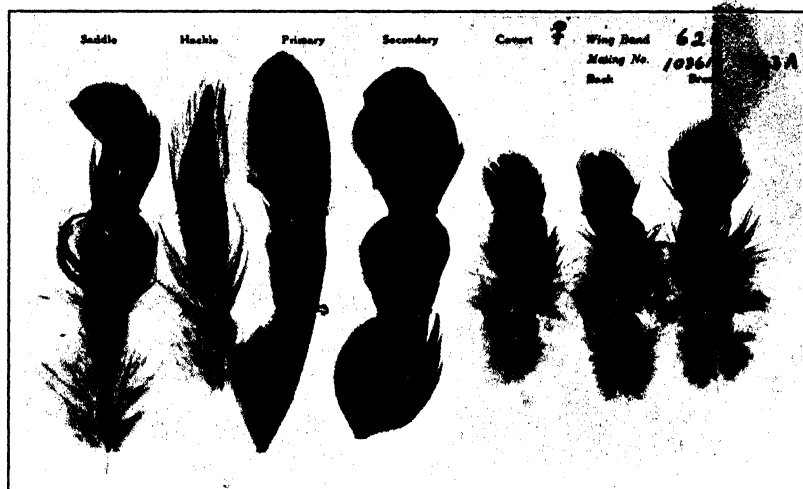


FIGURE 2.—A typical filled feather card containing sample feathers from seven representative sections of the plumage of a Rhode Island Red chicken

during the last two years were the classifications made from standards sufficiently definite to provide data of much value.

INHERITANCE OF DOWN COLOR

During the hatching seasons of 1925 to 1928 selections were carried out for the establishment of a light and dark down strain. Previous to the first season's matings no definite standard had been devised and the birds used for initiating the two strains had merely been described as individuals lighter or darker than the average. During the 1925 hatching season the chick down standard was first used; so that the matings beginning with 1926 were of birds of a known down grade. For the light strain, only birds whose down colors were of grades 1 and 2 were used, most of them being of the lighter grade. In the dark down selections all individuals used were of grades 4 and 5. Table 1 shows the results of the four years' selection. It will be seen that there is no sexual dimorphism with respect to down color, since the males and females in each generation show very similar distributions. Selection was much more effective in the light strain than in the dark one, and during the 1928 season a large

majority of the chicks in the light strain were of grade 1 with a limited number falling in grade 2. The last two years' results for the dark strain show a higher percentage of darker chicks than for the two preceding years, but in all generations there was a considerable number of grade 3 chicks. During the last two years' matings practically no chicks as dark as grade 3 were obtained in the light strain. Thus at the time of crossing of the two strains in 1927 there was practically no overlapping of the distributions of the light and dark strains.

TABLE 1.—Results of selection for light and dark down-color strains—Rhode Island Red chickens

SELECTION FOR LIGHT STRAIN (LIGHT BY LIGHT)

Year	Number of females of grade—					Number of males of grade—				
	1	2	3	4	5	1	2	3	4	5
1925.....	8	10	8	-----	-----	9	13	6	-----	-----
1926.....	7	28	18	-----	-----	8	29	14	-----	-----
1927.....	29	29	3	-----	-----	39	29	3	-----	-----
1928.....	36	8	-----	-----	-----	32	7	-----	-----	-----

SELECTION FOR DARK STRAIN (DARK BY DARK)

1925.....	-----	-----	9	4	-----	-----	-----	9	3	-----
1926.....	-----	1	37	25	2	-----	1	46	17	1
1927.....	-----	-----	29	27	1	-----	1	23	26	2
1928.....	-----	-----	11	7	1	-----	-----	9	9	2

TABLE 2.—Results of crossing of light and dark down-color strains—Rhode Island Red chickens

F₁ GENERATION

No.	Mating	Number of females of grade—					Number of males of grade—				
		1	2	3	4	5	1	2	3	4	5
1	Dark male by light female.....	-----	-----	33	2	-----	-----	3	32	5	-----
2	Light male by dark female.....	-----	9	73	7	-----	1	12	52	8	-----
	Total.....	-----	9	106	9	-----	1	15	84	13	-----

F₂ GENERATION

3	Male from mating 1 by female from mating 1.....	2	12	24	10	-----	7	3	15	5	-----
4	Male from mating 1 by female from mating 2.....	6	34	59	13	3	3	28	67	10	2
	Total.....	8	46	83	23	3	10	31	82	15	2
	Grand total, both sexes.....	18	77	165	38	5	-----	-----	-----	-----	-----

Reciprocal matings between the dark and light strains were made in 1927. The results of these crosses are shown in Table 2. Most of the F₁ offspring fall in grade 3, being intermediate between the two strains, but a few grade 2 and grade 4 chicks were also obtained.

The fact that the female chicks from the cross of light male by dark female are slightly lighter than those from its reciprocal might be taken to indicate that sex-linked factors are involved. However, the male offspring of this cross are also lighter, and this would not be the expected result from sex-linked inheritance. It would therefore appear that the difference between the reciprocals is more likely to be due to individual differences in the birds used as parents.

Table 2 also shows the results obtained for the F_2 generation produced in 1928. There are two series of F_2 's, one resulting from the mating of F_1 females of the cross light male \times dark females, and the other of females from the reciprocal cross. Both kinds of F_1 females were mated to the same F_1 male which was from the cross of a dark male by a light female. The F_1 individuals used to produce the F_2 generation were all of grade 3, this being the predominating color of that generation. The results for the F_2 generation show a distribution including the entire scale of grades. There was no significant difference between the down-color distribution of the offspring of the two lots of F_1 females. In Figure 3 are shown graphically the totals from Table 2 and the 1927 results from Table 1. The intermediate position of the F_1 graph would suggest a multiple-factor situation. However, the rapidity with which the dark and light strains were established by selection would indicate that the number of determining factors is not large. If we accept the variability occurring in the F_1 generation as an expression of the action of a single pair of genes and construct an estimated F_2 graph, we obtain one which approximates the F_2 results. The estimated graph was constructed by dividing the 303 F_2 individuals into three lots—one-fourth homozygous lights, one-fourth homozygous darks, and one-half heterozygotes. The estimated 76 lights (one-fourth of 303) were distributed over the range of color grades, shown by the 1927 light mating. In a like manner the same number of darks was distributed over the grades according to the range of the dark mating for the same year. The 151 heterozygous chicks were given a distribution modeled after the F_1 results of 1927. The estimated distributions (increasing in darkness of red from left to right) were as follows:

Grades.....	1	2	3	4	5
Lights.....	43	31	2	0	0
Darks.....	0	0	37	37	2
Heterozygotes.....	0	15	121	15	0

If the distributions for these classes are summed up, we find that the total estimated distribution corresponds relatively closely with the actual results:

Grades.....	1	2	3	4	5
Observed.....	18	77	165	38	5
Estimated.....	43	46	160	52	2

Although the estimated appears to fit fairly closely the observed distribution, the application of the χ^2 test³ for goodness of fit indicates that the difference here is significant and that a single pair of genetic factors does not fully account for the results. The χ^2 value was found to be 43.86, and the value of P , 0.000000. If the above results are examined it will be seen that the greatest differences in distribution come in the first two classes. If grades 1 and 2, and 3

³ YULE, G. N. AN INTRODUCTION TO THE THEORY OF STATISTICS. 5th ed. London.

and 4 are totaled in the estimated and obtained distributions, the fit is much better and shifts between these grades could easily have been due to errors in judgment. The χ^2 test is probably as reliable a test as can be applied to data of this kind, but in cases of extreme shifts between closely associated classes its degree of reliability can probably be questioned. In view of the above results, any attempt to estimate definitely the number of pairs of genetic factors involved in determining down color is probably unjustifiable. However, it appears to the writer that a single pair of autosomal factors will account for the major variations found in Rhode Island Red chick down color. Other minor factors probably operate, but they do not prevent the one pair of factors from accounting fairly satisfactorily

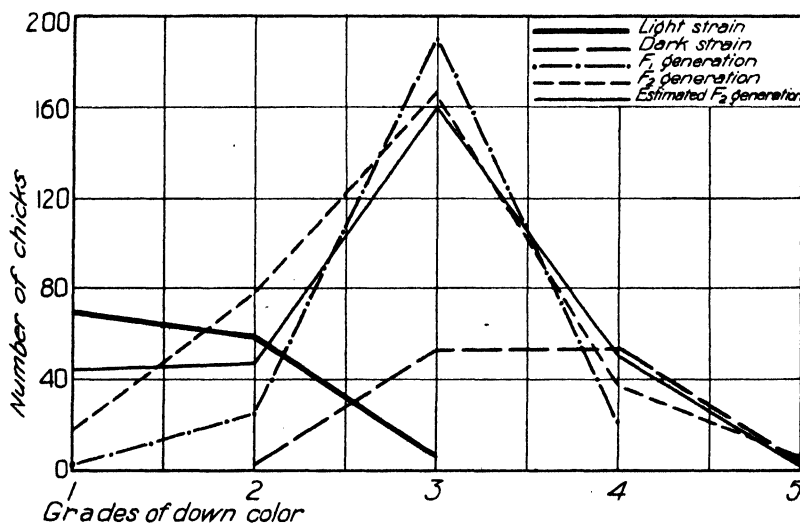


FIGURE 3.—Curves showing graphically the results of crossing light and dark color strains in Rhode Island Red chickens, drawn from figures given in Tables 1 and 2

for the results obtained. The F_1 generation shows neither condition to be dominant, since the distribution in this generation is intermediate between that of the two strains crossed.

COLOR STANDARD AND COLOR VARIATIONS

The ideal color for the Rhode Island Red breed as described in the American Standard of Perfection ⁴ (1923 edition) is as follows:

The plumage * * * should be rich red, except where black is specified, though the neck, wing bows, back, and saddle of the male should be a very lustrous, rich, brilliant red in order to comply with the Standard requirements of these sections. The less contrast between hackle, wing bows, back, breast, and body, the better, as an even shade of color throughout and an harmonious blending in all sections is desired. The shade of color should neither be so light a red as to suggest orange, nor so dark as to have a brownish or violet hue. An even, rich, brilliant red is preferred.

⁴ AMERICAN POULTRY ASSOCIATION. THE AMERICAN STANDARD OF PERFECTION ILLUSTRATED. A COMPLETE DESCRIPTION OF ALL RECOGNIZED VARIETIES OF FOWLS AS REVISED BY THE AMERICAN POULTRY ASSOCIATION AT ITS FORTY-SEVENTH ANNUAL MEETING AT KNOXVILLE, TENN., NINETEEN HUNDRED TWENTY-TWO. 1923 ed. 427 p., illus. n. p. 1923.

The primary wing feathers should be red like the rest of the plumage except the lower web which should be black with a red edging; the secondary wing feathers should have the black only in the upper web.

One of the common defects in the Rhode Island Red breed is that the general color is too light or too dark and the latter condition is usually accompanied by some black pigment on the surface. The breast is frequently much lighter than the rest of the plumage, this being a more common trait of females. The under color (the fluff portion of the feather which is hidden by overlapping) should match as closely as possible the general surface color, but this also varies considerably in shade, usually being lighter than the surface. The occurrence of black pigment in the under color, usually termed "smut," is a common defect. The appearance of white in the under color is also frequent, especially in males. The amount of black in wing feathers is frequently reduced or even may be entirely lacking.

The color of the eye varies from a dull gray to a reddish bay, the latter being the desired color. It was with these more common defects which were readily classifiable that the association of variations in down color was measured.

RELATION OF DOWN COLOR TO ADULT COLOR VARIATIONS

DOWN COLOR AND ADULT SURFACE COLOR

By surface color is meant the color of the back region, since the hackle and breast sections were classified separately. This is the section from which one would ordinarily get an impression of a bird's general color. The grades of surface color given in Tables 3 and 4 are those determined by comparison with the plumage standard already described. In this summary, as in all succeeding tables, the results of not only the down-color matings are given, but those of all Rhode Island Red matings for the period covered.

Since the males averaged somewhat darker than females with respect to surface color, it was necessary to tabulate the sexes separately. The results here confirm the statement made earlier that no sexual dimorphism exists with respect to down color, as the total distributions of the two sexes are very similar. In Tables 3 and 4 the darkness of the red in both down and adult plumage increases directly with the value of the grades. The coefficient of correlation⁵ of down and surface color of males (Table 3), was found to be 0.179 ± 0.020 . A value of so small a magnitude has, of course, no significance. In Table 4 are the data for the relationship in females of down and surface color. The coefficient of correlation was found to be 0.229 ± 0.016 , and, although higher than that of males, it is too small to be considered significant. Tables 3 and 4 show that the chicks of down-color grades 1 and 2 (both of which are very light) fall in all grades of plumage color. In other words, chicks of the lightest grades of down color may as adults be some of the darkest as well as some of the lightest-colored birds of the flock. However, the two darkest grades of down color (grades 4 and 5) produced adults all but two of which were grade 3 or darker. So, although light chicks may produce very dark adults, dark chicks seldom produce light adults. The intermediate down grade 3, like the lighter grades, produced adults having almost the full range of plumage shades.

⁵ BABCOCK, E. B., and CLAUSEN, R. E. GENETICS IN RELATION TO AGRICULTURE. 2d ed. New York. 79489—29—5

TABLE 3.—*Correlation between chick down color and adult plumage color (males only)*

Grades of adult color	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1		1				1
2	2	2	1			5
3	9	23	56	6	1	95
4	22	56	233	43	1	355
5	3	12	34	11		60
Total	36	94	324	60	2	

Coefficient of correlation 0.179 ± 0.020 .TABLE 4.—*Correlation between chick down color and adult plumage color (females only)*

Grades of adult color	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1		3	4			8
2	10	25	60	2		97
3	9	59	214	35		317
4	5	25	117	27	1	175
5		1	1			2
Total	25	113	396	64	1	

Coefficient of correlation 0.229 ± 0.016 .

DOWN COLOR AND BREAST COLOR

The plumage of the breast frequently is much lighter in color than the rest of the body. This is particularly true of females, and for that reason only females were considered in calculating the relation of down color to adult breast color. From Table 5 it is seen that no significant correlation exists, the coefficient being 0.238 ± 0.015 . The value of the coefficient is very similar to that obtained for females when the surface color other than that of the breast was considered.

TABLE 5.—*Correlation between chick down color and adult breast color (females only)*

Grades of adult breast color	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1	9	18	29			56
2	4	37	132	14		187
3	8	42	150	27	1	228
4	4	16	85	23	1	129
5		1	1			2
Total	25	114	397	64	2	

Coefficient of correlation 0.238 ± 0.015 .

DOWN COLOR AND EXCESS BLACK IN ADULT-PLUMAGE SURFACE COLOR

Particularly in strains of Rhode Island Reds which have been selected for a very dark shade of red there is a tendency to show varying amounts of black pigment on the surface. The appearance of black pigment anywhere except in the hackle of females and in wings and tail of either sex is undesirable. The males show black to a much lesser degree and for that reason only the data for females have been included in Table 6, where the relation of down-color variations to black in the plumage surface of adults is considered. This table shows the down-color distribution of those individuals showing black on the plumage surface in comparison with those free from it. The application of the χ^2 test indicates that the difference between these two distributions is not significant, since the value of P is slightly less than 0.029. In the application of the χ^2 test the method developed by Pearson⁶ for comparing two independent distributions of frequency from the same population but differing in N value, was used. This method was also used for calculating the χ^2 value from Tables 9 and 10. Since there is no significant difference between the down-color distributions for birds showing and lacking excess black on the surface, it may be said that the grade of down color does not affect the occurrence of black in this region.

TABLE 6.—Relation of down color to the occurrence of excess black in adult surface color (females only)

Item	Number of chicks with down color of grade—				
	1	2	3	4	5
Adults showing excess black	4	30	103	30	1
Adults lacking excess black	24	84	506	48	6
$\chi^2 = 8.77$ $P = 0.029$					

DOWN COLOR AND UNDER COLOR

The term "under color" as used by the poultry fancier refers to the color of the fluff sections of the feather. This is the basal portion of the feather which is usually hidden by overlapping. The under color was classified into four grades and its relation to chick down color is shown in Tables 7 and 8. The fluff sections of the feathers shown in Plate 2 present the four grades of under color, 1 to 4. Table 7 gives the data for males and Table 8 that for females. For each sex there is a significant positive correlation. For males the coefficient of correlation was found to be 0.478 ± 0.008 and for females 0.517 ± 0.006 . In each case these values may be considered significant, indicating that the lighter colored chicks have a tendency to develop into adults with under color which is lighter than the average. These results are clearly shown in Tables 7 and 8, for the chicks with down-color grades 1 and 2 mostly fell in adult grades 1 and 2 while the two darkest down-color grades seldom had adult plumage as light as grade 2.

⁶ PEARSON, K. ON THE PROBABILITY THAT TWO INDEPENDENT DISTRIBUTIONS OF FREQUENCY ARE REALLY SAMPLES FROM THE SAME POPULATION. *Biometrika* 8: 250-254. 1911.

This association could not have been the result of any bias of judgment on the part of the investigator, for he recorded the adult plumage grades without knowing what the down-color grade had been.

TABLE 7.—*Correlation between chick down color and adult under color (males only)*

Grades of adult under color	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1	5	4				9
2	24	49	90	4		167
3	6	37	214	53	2	312
4		6	20	4		30
Total	35	96	324	61	2	

Coefficient of correlation 0.478 ± 0.008 .

TABLE 8.—*Correlation between chick down color and adult under color (females only)*

Grades of adult under color	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1	4	9	1			14
2	18	72	147	8		245
3	3	30	218	42	1	294
4		3	30	15	1	49
Total	25	114	396	65	2	

Coefficient of correlation 0.517 ± 0.006 .

DOWN COLOR AND SMUT IN UNDER COLOR

The occurrence of smut in under color is a common defect in Rhode Island Reds. In Table 9 is shown the relation of smut to down color. For determining whether the grade of down color in any way affects the occurrence of smut, the birds were divided into two classes, those showing smut and those lacking smut. If down color influences the occurrence of smut, the two classes should show a difference in distribution for down color. Table 9 shows that the individuals exhibiting smut had lighter average down color than those lacking it. This was true for both sexes and the difference between the down-color distribution for the two lots was found to be significant from the application of the χ^2 test. Although many light chicks were free from smut and there were several of the darker grades which showed it, a difference in average down color of the two lots is evident.

TABLE 9.—*Relation of down color to the occurrence of smut in adult under color*

Item	Number of chicks showing down color of grade indicated—									
	Females					Males				
	1	2	3	4	5	1	2	3	4	5
Adults showing smut	18	39	64	7	1	15	15	24	8	
Adults lacking smut	14	79	351	69	2	22	74	305	51	2
	$\chi^2 = 50.08$					$\chi^2 = 37.73$				
	$P = 0.000000$					$P = 0.000000$				

DOWN COLOR AND WHITE IN UNDER COLOR

A common defect in Rhode Island Reds is the occurrence of white sections in the under color. This defect is found much more frequently in males than in females. The saddle is the section of the plumage where white more usually appears. The extent of white varies from a few feathers to large sections of the under color. Since the stock used for these studies showed white in under color somewhat infrequently in females, data are given for males only. (Table 10.) The comparison made was of individuals lacking and those having white in under color. The results show that the birds having white in under color had, as chicks, down which averaged considerably lighter than that of the birds that were free from this defect. To state the results in another way, the light-down chicks are more likely to show white in their under color as adults than the dark-down chicks. Of the 63 chicks having down of the color grades 4 and 5, only 9 showed white. Of the 127 chicks having down of color grades 1 and 2, 54 showed white and 73 showed none. The application of the χ^2 test for goodness of fit indicates that the difference obtained here is significant.

TABLE 10.—*Relation of down color to the occurrence of white in under color (males only)*

Item	Number of chicks showing down color of grade—				
	1	2	3	4	5
Adults showing white	18	36	56	9	2
Adults lacking white	17	56	269	52	
$\chi^2 = 47.7$ $P = 0.000000$					

DOWN COLOR AND BLACK IN WING

The amount of black in the primary and secondary wing feathers varies widely. In some cases black is entirely absent and in others it extends over practically the whole feather. These studies have shown that there is a very close correlation between the amount of black in the primary and in the secondary feathers. As has already been stated, the classification of grade of black in wing was determined by comparison with an arbitrarily graded standard, which is shown in Figure 1. To determine whether variations in shade of down color showed any relation to the amount of black in the wing, Tables 11 and 12 were made. Since the males show considerably more black in the wing than females, the records for the two sexes are presented separately. The data in these tables are for the primary wing feathers. The results on secondaries are not given since they are very similar. In neither case is the correlation significant, although in Table 12 there appears to be a slight tendency among the males for those in the darker wing grades to have darker down. The coefficient of correlation for males was 0.162 ± 0.028 and for females 0.133 ± 0.020 .

TABLE 11.—*Correlation between chick down color and amount of black in adult wing feathers (females only)*

Grades of black in adult wing	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1		18	34	8		69
2	25	52	210	29	1	310
3	4	37	125	21	2	184
4		2	5	31	12	52
5			4	13	7	20
6			1	1		7
Total	31	117	414	77	3	

Coefficient of correlation 0.133 ± 0.020 .TABLE 12.—*Correlation between chick down color and amount of black in adult wing feathers (males only)*

Grades of black in adult wing	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1	1	1	13	3		18
2	19	37	92	11	2	161
3	9	23	79	7		118
4	4	15	79	24		122
5	2	18	58	14		92
6	1		2	1		4
Total	36	94	323	60	2	

Coefficient of correlation 0.162 ± 0.028 .

DOWN COLOR AND EYE COLOR

The eye color most sought for by the poultry fancier is a brilliant reddish bay. There are found, however, varying shades of red in the eye of Rhode Island Reds. In some cases the red color may be entirely lacking, leaving the eye a light gray. The color referred to is that of the iris. In addition to variations in the amount of red there are differences in distribution of color in the different grades of eye color. The reduction of the red pigment is first seen at the margin of the pupil and the last traces are usually found at the outer edge of the iris. As was true of the plumage, the eye color was not graded until the 6-months age was reached, after which there appears to be very little change. Plate 2 shows from right to left the order of disappearance of red from the eye. Table 13 presents the relationship of down color to eye color. The two sexes showed the following distribution for eye color:

Males	5	55	177	183	93	5
Females	10	87	244	208	89	4

TABLE 13.—*Correlation between chick down color and adult eye color (both sexes)*

Grades of adult eye color	Number of chicks with down color of grade—					Total
	1	2	3	4	5	
1		2	6	7		15
2		19	101	21	1	142
3	21	80	270	50		421
4	28	80	242	38	3	391
5	19	28	113	21	1	182
6		1	7	1		9
Total	68	210	739	138	5	

Coefficient of correlation -0.123 ± 0.015 .

The grades become progressively darker from left to right. The results show no relationship between adult eye color and chick down color, since the coefficient of correlation -0.123 ± 0.015 is statistically insignificant.

DOWN STRIPING AND ADULT PLUMAGE COLOR

Although Rhode Island Red chicks are usually of a more or less uniform red color above, stripes occasionally occur on the back. These are ordinarily brownish in color and vary from definite stripes to small irregular spots. Chick A in Plate 1 shows striping. Striping occurs rather infrequently in the stock studies, so data are available on only a limited number of individuals. The records showed the striped chicks to be preponderantly females (29 females to 5 males). Although the sex ratio is distinctly aberrant, the difference may be a chance one resulting from the small numbers. The records for the 34 chicks that showed striping were studied in relation to the quality of the adult plumage. Much of the data on striping were taken before definite standards were used, and therefore did not lend themselves to uniform tabulation. However, an examination of the descriptions showed that the striped chicks did not differ from the average of other chicks with respect to adult surface color since the range was from light to dark individuals. The distributions for black in wing and eye color were also practically normal. The under color of the striped chicks was, however, found to be of very poor quality. The records for these chicks show that 6 had good red under color, 10 medium, and 18 poor. The quality here is far below the average for the nonstriped chicks. The explanation of this poor quality is found in the data on the occurrence of smut and white in the under surface. Twenty were listed as showing much smut, 8 some smut, 1 slight smut, and 5 no smut. Four showed much white, 10 some white, 5 slight white, and 15 no white. In all, 29 of the 34 showed some smut and 19 showed some white in the under surface. Considering all of the descriptions taken, less than 15 per cent of the females showed smut in the under surface, and the males showed much less. Of the striped female chicks over 85 per cent carried smut. Since so few females showed white in under surface no summaries were made for this sex in the study of white in under color. Although most of the striped chicks were females, over half of them showed some white in under color. One fact which should be taken into consideration is that striping is much more conspicuous in the lighter colored chicks and most of those listed as striped were of the lighter grades. Since it has already been shown that there is a relationship between down color and under color and also between down color and smut, the grade of down may also be a factor here. It can be said, however, that most of the striped chicks had poor quality of under color.

SUMMARY AND CONCLUSIONS

This study was conducted to determine whether Rhode Island Red chick down-color variations were heritable, and, if so, how they were inherited. It was also the purpose of the study to learn whether the variations found in down color bore any relation to adult plumage color variations. Any relationship found would, of course, be of

value in predicting, at hatching, the quality of adult plumage color to be expected. The relationship found between down and adult plumage color are purely physiological and not of the nature of genetic linkages.

It was found that variations in the shade of the down color are inherited and that it was possible to establish strains which bred relatively true for the dark and light red down. A single genetic-factor difference seemed sufficient to explain most variations encountered when the light and dark strains were crossed, neither condition being dominant.

The relationship of down-color variations to the following variations in adult plumage color were determined—general surface color, occurrence of black on the surface, breast color, under color, smut in under color, white in under color, black in the larger wing feathers, and eye color. The only significant correlation was found between the shade of down color and under color, and from this it was learned that a high percentage of the light-colored chicks developed into adults with light under color. In the same way most of the adults with the darker shades of under color showed dark-red down color as chicks. The lightest colored chicks also showed a tendency to develop into adults with under color showing much smut and white. Chicks showing conspicuous striping at hatching are likely to develop into adults with under color of inferior quality, usually carrying considerable smut and white in that section. No agreement was found between the shade of the adult surface color and down color. The chicks having the lightest shades of down might develop into very dark adults. It was found, however, that the dark chicks seldom produced adults of the lighter shades of red.

From a practical point of view, then, the light-colored Rhode Island Red chicks should be eliminated, primarily because of their effect upon the under color of the flock. If only individuals showing the darker shades of down are reared an improvement in under color may be expected. In order to eliminate smut and white from the under color, chicks showing striping should not be reared. If only chicks of the very darkest shades of down color are retained, the lighter colored adults will be avoided.

VARIETAL RESISTANCE OF SOYBEAN TO THE BACTERIAL PUSTULE DISEASE¹

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INTRODUCTION

The soybean, *Soja max* (L.) Piper, is susceptible to parasitic attack by a number of plant pathogenes. One of these, *Bacterium phaseoli sojense* Hedges, the causal agent of the bacterial pustule disease,² is widespread and occurs in a majority of the soybean fields in North Carolina. The organism overwinters in diseased leaf material³ and on seed from diseased plants.⁴ Seed treatments have served to delay the time when the disease became most abundant in a given season, but in no case has seed treatment given complete or even satisfactory control. The disposal of dead, diseased leaf material by plowing after harvest and the rotation of crops as generally practiced are undoubtedly valuable aids to control, but the disease has on a number of occasions developed in soybean test plots which had been planted with treated seed after an interval of at least one season in which no soybeans were grown on the land. In view of this situation it seemed desirable to determine if any of the various varieties or strains under cultivation showed more than usual resistance to the bacterial pustule disease. Accordingly, field observations were made and certain experiments were conducted in order to gather a body of information bearing definitely on this point. The data collected from these observations and experiments are presented in the following pages.

FIELD OBSERVATIONS

Observations were made at random in the field in the summers of 1925, 1926, and 1927 wherever a number of varieties of soybean were found growing together. The presence or absence of bacterial pustule was recorded and usually the relative amount of disease on the different varieties was estimated. The result of the observations made in the late summer of 1927 on plants growing in three variety tests are given in Table 1 as an example of the findings obtained in each of the three seasons.

This table shows that most of the varieties grown in these tests are susceptible to bacterial pustule. Such varieties as Dixie, Easy-cook, Ebony, Hahto, Hollybrook, Morse, and Peking showed the greatest amount of disease. Still others, as Austin, Virginia, Goshen, Haberlandt, Herman, Merko, and Pine Dell Prolific, were somewhat

¹ Received for publication Apr. 2, 1929; issued November, 1929. Published with the approval of the director of research as paper No. 33 of the Journal Series.

² HEDGES, F. BACTERIAL PUSTULE OF SOYBEAN. *Science* (n. s.) 56: 111-112. 1922.

³ HEDGES, F. A STUDY OF BACTERIAL PUSTULE OF SOYBEAN, AND A COMPARISON OF BACT. PHASEOLI SOJENSE HEDGES WITH BACT. PHASEOLI EPS. *Jour. Agr. Research* 29: 229-251, illus. 1924.

⁴ WOLF, F. A. BACTERIAL PUSTULE OF SOYBEAN. *Jour. Agr. Research* 29: 57-68, illus. 1924.

³ LEHMAN, S. G. STUDIES ON THE BACTERIAL PUSTULE DISEASE OF SOYBEAN. (Unpublished manuscript.)

⁴ WINTERS, R. Y. FIFTIETH ANNUAL REPORT OF THE NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION FOR THE FISCAL YEAR ENDED JUNE 30, 1928. STATISTICAL SUMMARY FOR YEAR ENDING DECEMBER 1, 1928. *N. C. Agr. Expt. Sta. Ann. Rpt.* 51: 60. 1929.

less severely infected but still showed considerable injury from the disease. Two varieties, Lexington and Columbia, were entirely free from bacterial pustule in the one test from which records for these varieties were secured.

TABLE 1.—*Relative amount of bacterial pustule disease observed on different varieties of soybean growing in the field in three localities of North Carolina, in 1927*

Variety	Relative amount of disease at—			Variety	Relative amount of disease at—		
	Willard	Raleigh	Moyock		Willard	Raleigh	Moyock
A. K.	(*)	Little	Much.	Laredo	Much.	Much.	Little.
Austin		Much.		Lexington		None	
Biloxi	Little	Little		Mammoth Yellow	Little		
Chiquita	do.	do.	Do.	Mammoth Brown	Much	Little	Much
Columbia		None		Merko		Much	
Dixie		Very much.		Morse		Very much.	
Easycook	Very much.			Otootan	Little	Much	Do
Ebony		Very much.		Old Dominion	do	Little	
George Washington		Much.		Peking		Very much.	
Goshen		do.		Pine Dell Prolific		Much.	Much.
Goshen Prolific		do.		Sooty	Little	do.	
Government		do.		Southern Prolific	Much	Little	
Haberlandt		do.	Do.	Tarheel Black	do.	do.	Much.
Hahto	Very much.			Tokyo	do.	do.	
Herman	Much.	Much.		Virginia	do.	Much	Do.
Hollybrook		Very much.		Wilson		Little	
				Yokoten		Much.	

* Varieties for which no data are recorded at a given place either had not been included in the test at that place or had reached maturity and shed their leaves at the time the observation was made.

Data such as those shown in Table 1, obtained by more or less random observation, are not very satisfactory as a basis for judging the disease resistance of different varieties of a given crop. For example, Mammoth Brown at Willard showed "much" disease while at Raleigh it showed "little." On the other hand, Otootan showed "little" at Willard and "much" at Raleigh. The reason for these differences in the amount of disease on the same variety growing in different locations may be conjectured to be due to any one of several causes, as, for example, to differences in amount of natural inoculation, or to variations in susceptibility of different strains of the host plant, or to differences in degree of maturity of the host at the time the organism causing the disease was being disseminated. In order to obviate these differences to as great an extent as possible it seemed desirable that further data should be obtained from tests in which the varieties used were artificially inoculated with an abundance of the causal organism. By this method data could also be secured on a larger number of varieties. Two such tests, one in the greenhouse and one in the field, were made.

GREENHOUSE VARIETY TEST

In the greenhouse test 10 seeds of each variety were planted at a marked location in sandy soil in the greenhouse bench. After the plants had attained suitable size they were inoculated on several occasions by spraying with a water suspension of young cultures of

Bacterium phaseoli sojense previously isolated from a typical pustulate lesion taken from a leaf of the Biloxi variety. Just previous to each inoculation the soil of the bench was thoroughly wetted, and immediately following inoculation the bench was covered with a muslin cloth kept moist by repeated sprinkling over a period of three to four days, at the end of which time the cloth was removed. The seeds were planted on December 20, 1927, and the first inoculation was made on January 24, 1928. Subsequent inoculations were made on January 31, February 13, March 14, March 16, and March 20. The varieties of soy beans used in the test and the results obtained are recorded in Table 2.

TABLE 2.—Relative resistance of different soybean varieties to infection by *Bacterium phaseoli sojense* in a greenhouse variety test, 1928

Variety	Ratio of number of plants infected to number inoculated, intensity of disease and coefficient of susceptibility, on dates indicated							Group No.
	Feb. 21		Mar. 30		Apr. 11			
	Ratio	Intensity	Ratio	Intensity	Ratio	Intensity	Coefficient	
Austin	1:8	1		(a)				
A. K.	3:9		3:9	a b 1	7:9	1	2.05	2
Black Eyebrow	0:6		c 4:5	b 1	5:5	2	5.00	3
Biloxi	4:9	2	c 7:8	b 1	8:8	2	5.00	3
Chestnut	0:6		0:6	(d)	4:6	1	1.65	2
Columbia	0:1		0:1	(b)	0:1		.00	1
Chiquita	0:7		3:7	c 2	7:7	2	5.00	3
Dixie	0:6		c 1:5	b 1	4:5	2	4.00	2
Ebony	0:7		0:7	(d)	3:7	1	1.07	2
Goshen Prolific	2:10	2		(a)	9:9	5	12.50	4
George Washington	0:7		0:7	(b)	6:6	1	2.50	2
Government	0:4		0:4	(c)	0:4		.00	1
Haberlandt	5:10	5	10:10	c 8	10:10	8	20.00	4
Hamslet	0:4		1:4	c 1	3:4	1	1.87	2
Hollybrook	7:8	6	8:8	b 5	8:8	5	12.50	4
Habaro	1:7	1	c 2:6	d 1	6:6	1	2.50	2
Johnson	4:5		c 4:4	c 1	4:4	1	2.50	2
Lexington	3:8	1	3:3	b 1	3:3	2	5.00	3
Laredo	0:7		0:7	(b)	5:8	1	1.55	2
Morse	4:9	1	4:8	b 1	9:9	2	5.00	3
Merko	2:10	3	2:10	(a)	7:10	1	1.75	2
Mammoth Yellow	0:7		6:7	b 2	7:7	2	5.00	3
Manchu	0:7		0:7	(c)	5:7	1	1.77	2
Mammoth Brown	0:7		c 4:5	c 1	5:5	3	7.50	3
Old Dominion	0:6		0:6	(c)	0:6		.00	1
Ootootan	0:9		6:9	b 1	8:9	2	4.40	2
Peking	0:8		0:8	a (b)	0:8		.00	1
Pine Dell Prolific	1:7	1	1:7	b 1	6:6	2	5.00	3
Southern Prolific	0:9		0:9	(c)	8:9	1	2.20	2
Tarheel Black	3:10	3	7:10	b 5	8:10	5	10.00	4
Tarheel Black	8:9	3	8:9	b 1	9:9	2	5.00	3
Tokyo	0:7		3:7	b 1	6:7	2	4.25	2
Virginia	10:10	5		(a)			12.50	4
Wilson	7:9			(a)				
Wilson Black	1:8	1	2:8	(a)				
Yokoten	0:6		6:6	b 3	6:6	3	7.50	3
54610	1:7	1	1:7	(b)	7:7	1	2.50	2
37261	0:9		c 6:7	b 2	7:7	2	5.00	3
36906-A-7-10	0:6		0:6	b 3	6:6	3	7.50	3
36906-A-7-5-1	0:8		1:8	b 1	6:8	1	1.87	2

^a Top leaves badly injured from becoming too dry.

^b Growing rapidly, foliage tender.

^c Remainder of plants died since last record was taken.

^d Stunted, not growing properly, apparently because of unfavorable length of day.

^e Growing but less rapidly than varieties marked b, and possessing less succulent leaves.

^f Authors uncertain of correctness of this name.

In this table infection records are presented in two columns under three different dates. The fractions in the first column are the ratios of diseased to inoculated plants, the denominator being the number of plants inoculated, the numerator the number which became infected on one or more leaves. The numbers in the second column under each date are intended to convey an idea of the intensity of the disease as indicated by the estimated relative number of lesions per leaf, the larger integers indicating proportionally larger numbers of lesions. The coefficients of susceptibility shown in the third column under date of April 11 were calculated for each variety by the following formula:

$$\frac{\text{Number of infected plants (or leaves)}}{\text{Number of inoculated plants (or leaves)}} \times \text{intensity} \times 2.5$$

By this formula the two factors used to measure susceptibility are combined in a single multiple for the purpose of facilitating comparison between varieties. The constant 2.5 is used only in Table 2. The range of the values used to indicate intensity of infection in Table 3, which is to be compared with Table 2, is 2.5 times as great in the former as in the latter; hence the true coefficients in the former are 2.5 times as great as in the latter for the same varieties. Introducing this factor 2.5 into the formula used for Table 2 and omitting it for Table 3 removes this difference and greatly facilitates comparison of the results recorded in the two tables. The different varieties are ranked and assigned a group number, using the coefficients of susceptibility as a basis of comparison. The varieties having the lowest coefficients of susceptibility are placed in Group 1; those with highest coefficients, in Group 4. The different groups thus arbitrarily established are shown in Table 4.

During the interval between the first inoculations in the greenhouse variety test and the time of taking the first data on February 21, the temperature of the greenhouse was too low for a high percentage of infection to occur. However, a comparatively small number of infections were found on the most susceptible varieties. By March 30, three additional inoculations having been made in the meantime, a considerable increase in the number of diseased plants had occurred. The proportion of diseased to healthy plants was still low, however. Because there was so little disease up to March 30, the data recorded on that and the previous date are not used in determining coefficients of resistance, but are presented to show the slow increase of disease with ameliorating temperature conditions and to corroborate the findings based on the data taken April 11. For several days previous to April 11, when the last records were taken, the daytime temperatures had been uniformly high and the nights warm, resulting in development of the disease on nearly all the plants. The grouping of the varieties in relation to resistance is based on the data taken on this date.

TABLE 3.—Relative resistance of different soybean varieties to infection by *Bacterium phaseoli sapineae* in field experiment, 1928

Variety	Ratio of number of plants or leaves infected to number inoculated, intensity of disease, and coefficient of susceptibility on dates indicated						Average coefficient	Group No.
	August 1			September 24				
	Ratio (plants)	Intensity	Coefficient	Ratio (leaves)	Intensity	Coefficient		
Austin	38:38	8	8.00	(*)			8.0	3
A. K.	54:58	4	3.72	1.0			6.8	3
Aksarben	21:23	4	3.64	1.0	10	10.0	6.8	3
Black Eyebrow	54:54	2	2.00	1.0	8	8.0	5.0	3
Biloxi	59:59	8	8.00	.75	5	3.75	5.8	3
Barchet	11:15	4	2.92	.8	5	4.0	3.4	2
Chiquita	30:30	5	5.00	.9	5	4.5	4.7	2
Chestnut	50:50	10	10.00	1.0	10	10.0	10.0	4
Columbia	2:22	1	.09	Trace			0.9	1
Dixie	18:19	5	5.00	1.0	15	15.0	10.0	4
Ebony	45:45	15	15.00	1.0	12	12.0	13.5	4
Easycook	13:13	1	1.00	.5	2	1.0	1.0	2
George Washington	22:22	8	8.00	1.0	15	15.0	11.5	4
Goshen Prolific	77:77	20	20.00	.8	10	8.0	14.0	3
Government	43:44	5	5.00	.8	5	4.0	4.5	2
Herman	16:16	8	8.00	1.0	18	18.0	13.0	4
Hamshet ^a	4:4	8	8.00	(*)			8.0	3
Haberlandt No. 12	63:63	5	5.00	1.0	10	10.0	7.5	3
Hollybrook	55:55	10	10.00	1.0	18	18.0	14.0	4
Hubaro	28:28	5	5.00	1.0	10	10.0	7.5	3
Hoosier	30:30	10	10.00	1.0	15	15.0	12.5	4
Hamilton	4:4	4	4.00	1.0	15	15.0	9.5	3
Hahto	14:14	2	2.00	.8	5	4.5	3.2	2
Ito San	11:11	2	2.00	(*)			2.0	2
Johnson	25:25	8	8.00	10.0	10	10.0	9.0	3
Laredo	30:30	5	5.00	.6	2	1.2	3.1	2
Lexington	37:37	5	5.00	1.0	12	12.0	8.5	3
Mammoth Brown	42:42	8	8.00	1.0	10	10.0	9.0	3
Morse	71:72	5	5.00	1.0	8	8.0	6.5	2
Manchu	32:32	5	5.00	1.0	8	8.0	6.5	3
Merko	42:42	10	10.00	1.0	20	20.0	15.0	4
Mammoth Yellow	56:56	5	5.00	1.0	5	5.0	5.0	3
Midwest	27:30	8	7.20	1.0	15	15.0	11.1	4
Mandarin	7:8	1	.87	(*)			.8	1
Medium Green	20:20	4	4.00	1.0	15	15.0	9.5	3
Mikado	22:23	2	2.00	1.0	10	10.0	6.0	3
Minsoy	5:5	2	2.00	1.0	20	20.0	11.0	4
Otootan	18:18	10	10.00	1.0	10	10.0	10.0	4
Old Dominion	19:19	3	3.00	1.0	5	5.0	4.0	2
Pine Dell Prolific	46:46	8	8.00	1.0	18	18.0	13.0	4
Peking	28:30	4	2.82	1.0	10	10.0	6.4	3
Southern Prolific	52:52	5	5.00	1.0	15	15.0	10.0	4
Tokyo	52:52	5	5.00	.9	5	4.5	4.7	2
Tarheel Black	35:47	1	.74	.5	2	1.0	.8	1
Virginia	77:77	15	15.00	1.0	12	12.0	13.5	4
Wilson	72:72	10	10.00	1.0	10	10.0	10.0	4
Wilson Black	35:36	5	5.00	.9	5	4.5	4.7	2
Wilson Five	6:34	2	.34	.8	5	4.0	2.1	2
Wea	9:9	2	2.00	(*)			2.0	2
Yokoten	24:24	5	5.00	1.0	20	20.0	12.5	4
35906-A-7-5-1	26:26	5	5.00	1.0	10	10.0	7.5	3
35906-A-7-10	17:17	5	5.00	1.0	5	5.0	5.0	3
37261	43:43	8	8.00	1.0	15	15.0	11.5	4
54610	12:12	5	5.00	1.0	5	5.0	5.0	3
Yokoten ^a	3:3	4	4.00	.8	2	1.6	2.8	2

* This variety had matured and shed its leaves at this date.

^b Authors uncertain of correctness of this name.

TABLE 4.—Groups into which soybean varieties were classified on a basis of the coefficient range and their relative resistance to infection by *Bacterium phaseoli sojense*

Group No.	Coefficient of susceptibility	Resistance
1	Less than 1.....	High.
2	1 to less than 5.....	Moderately high.
3	5 to less than 10.....	Moderately low.
4	10 to 20.....	Low.

Forty varieties and selections were included in this test. Of this number 4, Columbia, Government, Peking, and Old Dominion, are placed in Group 1 for high resistance; 16, in Group 2 for moderately high resistance; 12, in Group 3 for moderately low resistance; 5, in Group 4 for low resistance; and 3 are given no group number because of insufficient data. It is probable, as indicated in Table 1, that the variety Peking in Group 1 would have fallen into Group 2, 3, or 4 had not the top leaves been injured by severe drying about the time temperatures in the greenhouse were becoming favorable for infections to occur. The plants of the varieties Old Dominion and Government were 8 to 10 inches tall on March 30. At that time they possessed four and five trifoliolate leaves, respectively, and were growing but not so rapidly as some other varieties. It is probable that under conditions more favorable for growth and for occurrence of infections these plants would have fallen in Group 2 or 3. Only one plant of the variety Columbia came up. On March 30 this plant was growing rapidly, having produced nine trifoliolate leaves on a stem 24 inches long. The tender succulent character of this plant was very favorable for infection, the success of which was very probably thwarted by the factor of inherent resistance. In general, the plants falling in Groups 3 and 4 were growing well and possessed tender foliage. On the other hand, some of the varieties placed in Group 2, notably Chestnut, Ebony, Habaro, and Manchou, showed a stunted condition accompanied by very slow growth and early pod development, due supposedly to a photoperiod unfavorable for the best development of these varieties at this season of the year. Had conditions been favorable for more rapid growth, such for instance as occurs in the field in the summer months, these four varieties probably would have shifted to a place with the less resistant varieties in Group 3 or Group 4.

FIELD VARIETY TEST

The seeds used in the field experiment were planted in sandy loam soil on bottom land adjacent to a small creek. Seeds of most of the varieties were planted on June 18, 1928; the remainder, on July 2.⁵ The plants were inoculated by spraying them on several occasions with suspensions of *Bacterium phaseoli sojense* of the same strain used in the greenhouse experiment described above. Late afternoon or evening and, as often as possible, rainy days were chosen as the most fitting time for applying the bacteria to the plants. The first inoculation was made in the evening of July 12, a cloudy, showery

⁵ Acknowledgment is made of the kind cooperation of William J. Morse, of the U. S. Department of Agriculture, who furnished seed of 15 of the varieties used.

day. This inoculation was confined to the varieties planted on June 18, since these plants had two to three trifoliate leaves apiece, while some of the varieties planted on the later date, July 2, were just coming through the ground. The second inoculation was made on July 17, at which time all the varieties were sprayed with the bacterial suspension. Other inoculations were made on one subsequent date in July and on three in August.

As a means of testing the virulence of the bacterial suspension used for the several inoculations, a pot of plants of the Herman variety was inoculated each time and kept for a few days under a bell jar in the greenhouse. In each instance the disease developed abundantly on the check plants in the greenhouse.

On August 1, and again on September 24, the plants in the field were examined in order to determine the amount of disease present. The data obtained are recorded in Table 3. With the exception of two varieties, Columbia and Wilson Five, all or practically all of the plants of each variety in the test were diseased by August 1. By September 24 the plants of many of the varieties had become so large and tangled that counting was not feasible, and it was thought that an estimation of the percentage of diseased leaves would serve as a better basis of comparison. These data, originally taken as percentage of diseased leaves, are recorded not in terms of percentage but as the equivalent decimal fraction (column 5). This decimal expresses the ratio of diseased to inoculated leaves. The coefficient of susceptibility is calculated for each variety at each time of taking the data. The average of the two coefficients is employed to determine the rank or group number of each variety using as a basis the scale given in Table 4.

Fifty-five varieties were tested in this experiment. Of this number, 3 fall into Group 1 for high resistance; 13 into Group 2 for moderately high resistance; 22 into Group 3 for moderately low resistance; and 17 into Group 4 for low resistance. The three most resistant varieties, as indicated by this test, are Columbia, Mandarin, and Tarheel Black. On September 24, when the second set of data were taken, the variety Mandarin was mature and had shed all its leaves. It is quite possible that this variety would have fallen into Group 2 if a second set of data had been taken previous to the shedding of its foliage. In regard to the proper placing of the variety Tarheel Black, the field and the greenhouse inoculation experiments are not in agreement. In the field this variety, as noted above, fell into Group 1, while the results obtained in the greenhouse experiment placed it in Group 4. General field observations, as recorded from time to time (Table 1), indicate that Tarheel Black is not highly resistant to bacterial pustule but should be put in Group 2 or possibly Group 3 rather than in Group 1.

A summary of the results of the greenhouse and field tests is presented in Table 5. Of the 16 varieties placed in Group 2 in the greenhouse test, only 2, Laredo and Tokyo, fall into that group in the field test, the remaining 14 falling equally in Groups 3 and 4. Seven of the 13 varieties in Group 2 of the field test were not included in the greenhouse test; 2 were from Group 1, 2 from Group 2, 1 from Group 3, none from Group 4, and 1 was not classified. Of the 12 varieties in Group 3 of the greenhouse test, 2 shift to higher groups in the field tests, 7 remain in Group 3, and 3 shift to Group 4. Only 3 of the 5

varieties in Group 4 of the greenhouse test shift to higher groups in the field, while of the 17 in Group 4 in the field test, 7 came from Group 2 and 3 from Group 3 of the greenhouse test. There is evident a general shifting of varieties from groups of high resistance into groups of lower resistance with but little shifting in the opposite direction when the experiment is transferred from the greenhouse to the field. Such a shift is to be expected when one considers that the greenhouse experiment was conducted in winter and early spring when temperature and other conditions were not so favorable for infection as in the field in summer.

TABLE 5.—Summary of results of greenhouse and field tests on the resistance of different soybean varieties to infection by *Bacterium phaseoli sojense*

Group No.	Number of varieties in each resistance group		Varieties which are same in each experiment		Number of varieties in greenhouse test shifting to other groups in field test	
	Greenhouse experiment	Field experiment	Number	Name	To higher resistance group	To lower resistance group
1	4	3	1	Columbia	0	3
2	16	13	2	Laredo	0	14
				Tokyo		
3	12	22	7	Black Eyebrow, Biloxi, Morse, Lexington, Mammoth Yellow, Mammoth Brown, 35906-A-7-10	2	3
4	5	17	2	Hollybrook (Virginia)	3	
Total	40	55	12		5	20

* Includes three varieties not classified.

Both the field and greenhouse tests indicate that the variety Columbia holds first place among the varieties tested for resistance to bacterial pustule. This finding is in harmony with the observation recorded in Table 1, in which Columbia was free from bacterial pustule when varieties in near-by rows showed high infestation. Of the other three varieties, Government, Old Dominion, and Peking, which fell in Group 1 in the greenhouse variety test, Government and Old Dominion fall into Group 2 and Peking in Group 3 in the field test.

The varieties Hollybrook and Virginia fall in Group 4 in both experiments. This fact would seem to mark them as the most susceptible of the varieties tested. However, notes taken in the field but not shown in Table 3 indicate that the varieties Yokoten, Minsoy, Merko, and Pine Dell Prolific were injured quite as badly as Hollybrook and perhaps more than Virginia. Lesions were very numerous on the leaves of all these varieties and a large proportion of the leaf tissue was killed by confluence of diseased areas.

GREENHOUSE TEST WITH COLUMBIA

As a further test of the resistance of the variety Columbia to the bacterial pustule disease, young plants growing in pots in the greenhouse and having one trifoliate and two unifoliate leaves open were

inoculated on January 30, 1929, by rubbing their upper and lower surfaces with a heavy suspension of the pustule organism. The plants were immediately placed under bell jars and kept there for four days in order to maintain a condition of high relative humidity favorable to infection. During the infection and incubation period the greenhouse was kept at a temperature previously determined to be favorable for development of the disease. As a check on the virulence of the cultures used, young plants of the Herman variety were inoculated by the same method and with the same bacterial suspension used on Columbia. Still another check of Herman was inoculated in the same manner but with a nonparasitic bacterium to test the effect of such mechanical injuries to the leaves as might result from handling and rubbing them in the process of inoculation. Both checks of the Herman variety were subjected to the same conditions of humidity and temperature as those of the variety Columbia.

At the end of nine days no infections could be detected on Columbia, but infections on Herman plants inoculated with *Bacterium phaseoli sojense* were very numerous, and some of the leaves were dying because of the great number of lesions. The bacteria entered quickly and produced rapid injury at places of mechanical abrasion, but large numbers of lesions resulted from stomatal infection also. Some mechanical injury was apparent on the Herman plants inoculated with the nonparasitic bacterium, but no symptoms typical of lesions of bacterial pustule developed about these injuries nor did any of the leaves die. Likewise, mechanical abrasions were apparent on the plants of the Columbia variety, but no bacterial infections occurred at any time at the points of injury on these plants, not did any of the leaves die from mechanical or pathological effects resulting from the inoculation.

By the sixteenth day, however, a comparatively small number of infections (about 50 in all) had occurred on the variety Columbia. All of these were on trifoliate leaves, the great majority occurring on two small areas of the 12 trifoliate leaflets inoculated. No infections were present on the unifoliate leaves of Columbia, while on the corresponding leaves of the Herman variety lesions were very numerous. From this it appears that the very young leaves of the Columbia variety are susceptible to infection under especially favorable conditions and that they lose this susceptibility more quickly than the leaves of Herman.

A very obvious difference was apparent in the size and appearance of the lesions on the leaves of the Columbia and Herman varieties. The lesions produced by the bacterial pustule organism are normally characterized by the development of a pustule the base of which is circumscribed by a yellow halo. The pustule usually soon ruptures or dries down and a brown or reddish spot appears. This brown spot may increase considerably in size, but the yellow halo is always an evident and often the most conspicuous feature of the lesion. On the variety Columbia, in the experiment recounted above, only the faintest suggestion of a yellow halo could be seen about some of the pustules, while about most of them no evidence of a halo could be distinguished, the spot being no wider than the base of the pustule itself. On the leaves of Herman, however, the yellow halo was strongly evident, the brown necrotic areas in the centers of the lesions were broader, and the estimated average total width of the lesions

was three to four times that of the lesions on Columbia. This practically complete suppression of the yellow halo and the smaller size of the lesions may be taken as another indication of resistance on the part of the variety Columbia.

DISCUSSION AND CONCLUSIONS

Random field observations and more carefully executed artificial inoculation tests in the greenhouse and in the field have yielded certain data indicative of the relative resistance of 56 different varieties of soybean to the bacterial pustule disease. Lists of the varieties which possess highest, intermediate, and lowest resistance, as judged by the writer from general field observation and experimental tests, are given below. The varieties in each list are arranged in the order of decreasing resistance, the least resistant coming last.

Highest	Intermediate	Lowest
Columbia	Laredo	Hoosier
Mandarin	Chiquita	Midwest
Old Dominion	Mammoth Yellow	Medium Green
	Tarheel Black	Virginia
	Biloxi	Herman
	Otootan	Haberlandt
	Goshen Prolific	Pine Dell Prolific
	Southern Prolific	Hollybrook
		Minsoy
		Merko
		Yokoten

Minsoy, Hollybrook, Merko, and Yokoten are probably the most susceptible. Herman, Virginia, Pine Dell Prolific, and Midwest are also highly susceptible. Aside from Herman and Virginia, the varieties most commonly cultivated in North Carolina, namely, Laredo, Otootan, Mammoth Yellow, Biloxi, and Tarheel Black, are intermediate in resistance.

The variety Columbia is highly resistant to the particular strain of the bacterial pustule organism used in the tests described in this paper. This high resistance is indicated by the results of the greenhouse and the field variety tests. Although only 1 plant of this variety was available for use in the greenhouse variety test, this plant grew rapidly and was in a tender condition favorable for infection during the whole period of the test. It had produced nine leaves on a stem 24 inches long and was larger than the plants of any other variety on March 30, when the last disease records were taken. Because of its large size and numerous leaves it could not have escaped a liberal application of inoculum. That this variety is not completely resistant or immune to bacterial pustule is shown by the occurrence of lesions on 2 of the 22 plants examined in the field on August 1, and on the plants of this same variety inoculated in the second greenhouse test on January 30, 1929. In this second greenhouse test conditions very favorable for infection were maintained, with the result that some infections did occur on Columbia. However, the amount of disease on Columbia was small compared with that on Herman. The very young leaves of Columbia are susceptible under conditions very favorable for infection, but they lose this susceptibility much earlier than those of more susceptible varieties. This is indicated by the occurrence of numerous lesions on the unifoliate

leaves of Herman, while the corresponding leaves of Columbia remained free. The relatively small number of lesions which did occur on Columbia in the second greenhouse test, the slowness with which they appeared, and the nearly complete suppression of the customary yellow halo are indications of the relatively high resistance of this variety.

The same strain of *Bacterium phaseoli sojense* was used in all the tests described in this paper. This strain (S. B. P. 8 Col. A) was isolated on August 28, 1927, from a young lesion on a leaf of the Biloxi variety. It will be of interest to learn whether this variety is equally resistant to all strains of this organism.

According to Piper and Morse,⁶ the variety Columbia was introduced in to America from Paotingfu, China, in 1908. It is a green-seeded variety which produces a rather stout erect growth and bears both purple and white flowers. It matures in about 125 days. Columbia is not grown on the farms of North Carolina, but it is hoped that its resistance will be found to encompass all strains of the bacterial pustule organism, and that it may be used as a parent in the work of combining the quality of high resistance to bacterial pustule with the desirable characters of the more popular varieties of soybeans now being grown.

SUMMARY

Certain observations have been made relative to the natural occurrence of the bacterial pustule disease of soybean in variety tests in the seasons of 1925, 1926, and 1927. A record of the relative amounts of disease occurring on 33 different varieties observed in 1927 is given.

Artificial inoculation tests were made in 1928 in which 40 varieties of soybeans growing in the greenhouse and 55 varieties planted in the field were sprayed on several occasions with heavy suspensions of *Bacterium phaseoli sojense*. Columbia was found to possess greater resistance to infection by this bacterium than any other variety used in these tests.

Infections did occur on Columbia when inoculated plants were kept under especially favorable conditions, but the lesions developed more slowly, were smaller and fewer in number than on resistant varieties, and development of the halo was almost completely suppressed.

The varieties Hoosier, Midwest, Medium Green, Virginia, Herman, Haberlandt, Pine Dell Prolific, Hollybrook, Minsoy, Merko, and Yokoten are the least resistant of the varieties tested. The more commonly cultivated varieties Laredo, Chiquita, Mammoth Yellow, Tarheel Black, Biloxi, Ootootan, Gosehn Prolific, and Southern Prolific are intermediate in resistance.

⁶ PIPER, C. V., and MORSE, W. J. THE SOYBEAN. 329 p., illus. New York. 1923.

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A PATHOLOGICAL FEATURE OF FLEA-BEETLE INJURY OF POTATO TUBERS¹

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INTRODUCTION

The potato flea beetle (*Epitrix cucumeris* Harr.) (5)² has long infested potatoes in northern Colorado. Since 1904 the annual loss caused by the insect in that section has usually amounted to \$100,000 or more (2). The damage caused by the feeding of the adult beetles on the foliage is considerable, but the losses caused by the feeding of the larvae on the tubers are unquestionably of more importance. Under sales competition and grading rules the potatoes suffer, with losses to grower and dealer and to the reputation of the district generally. From year to year the damage has varied in severity. Efforts to control the beetle have been only partially successful. A study of the beetle damage to tubers grown in pathological experiments led to the belief that some additional factors might be responsible for some of the severe injury usually attributed to flea beetles.

REVIEW OF LITERATURE

The literature dealing with the flea beetle is extensive and voluminous. No effort has been made to review it all, as studies of morphology and life history are of no concern here. The statements concerning damage to the tubers are brief and consistent. Stewart (13) stated that the larvae made "pimples" on the tuber surface and developed "slivers" in the flesh. Johannsen (3), Webster (14), and Britton (1) reported the same type of injury. None of them, however, mention long tuber scars or worm tracks. Recently Hoerner and Gillette (2), in a competent life-history study, mentioned the worm track but briefly.

EXPERIMENTAL FIELD CONDITIONS

At the Colorado Potato Experiment Station, Greeley, Colo., potato diseases are being studied. For some years the flea beetle has been a growing evil, damaging vines and tubers and spoiling the experimental results. In an effort to combat the beetle, all experimental plots, regardless of the purpose for which they were intended, were sprayed with a 5-5-50 Bordeaux mixture to which was added 2 pounds of Paris green per 100 gallons. In times past the beneficial results of

¹ Received for publication Mar. 30, 1929; issued December, 1929.

² Reference is made by number (italic) to "Literature cited," p. 814.

this treatment have varied. In 1928 the field was sprayed only twice because a hail on July 19 stripped the vines of leaves, broke the stems and branches, and practically ruined the field for its original purpose. After that date spraying was not considered worth while. By that time, however, the beetles should have laid their eggs (2).

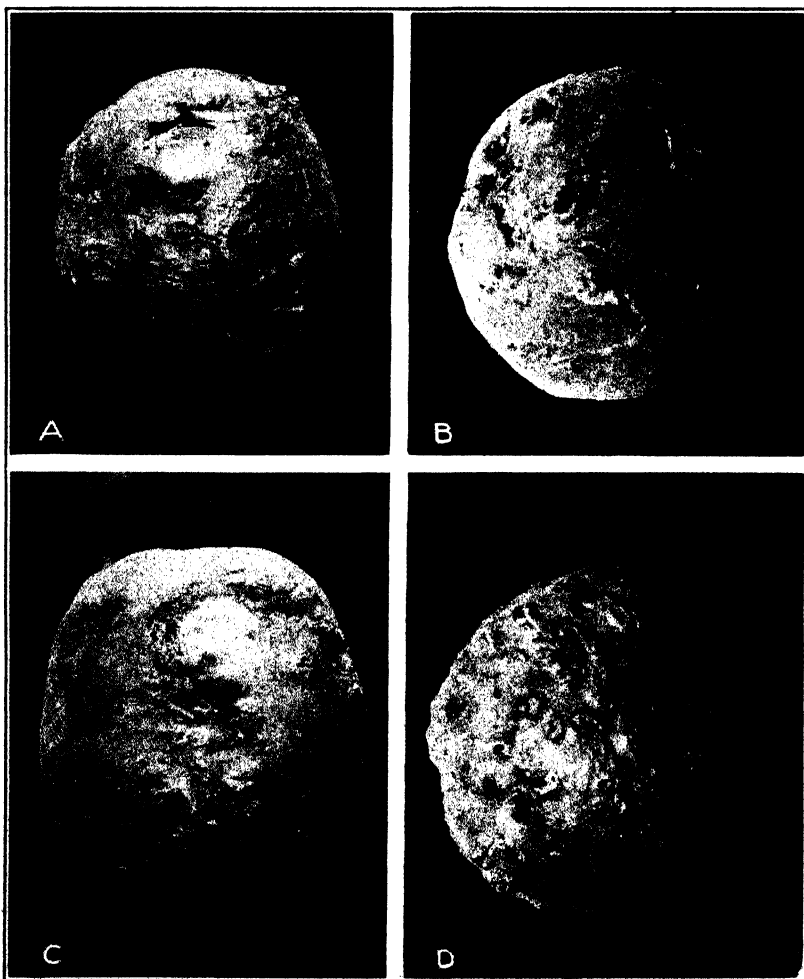


FIGURE 1.—A-C, Tubers showing slight scab-infected worm-track and pimple injuries. The seed potato from which A was grown was treated with graphite, a substance nonpoisonous to pathogenic organisms. The cut seed potato from which B was grown, and the whole seed potato from which C was grown, were untreated. The worm tracks in B were infected with both *Actinomyces* and *Rhizoctonia*. D, Scab-infected tuber grown from an untreated whole parent tuber and showing typical pimple development

The experimental field was originally intended to be used for testing seed treatments to control *Fusarium* blight. The seed potatoes were treated in different ways. After cutting, parts of the seeds were dipped in each of three organic-mercury compounds; and, prior to cutting, other seeds were soaked one and one-half hours in 1:1,000

mercuric chloride. Cut seed dusted with graphite, untreated whole seed, and untreated cut seed as controls completed the experiment. All the seed potatoes were of the Bliss Triumph variety from a single source.

During the season the field was irrigated but twice. Ordinarily five or six irrigations would have been given. Unusually severe

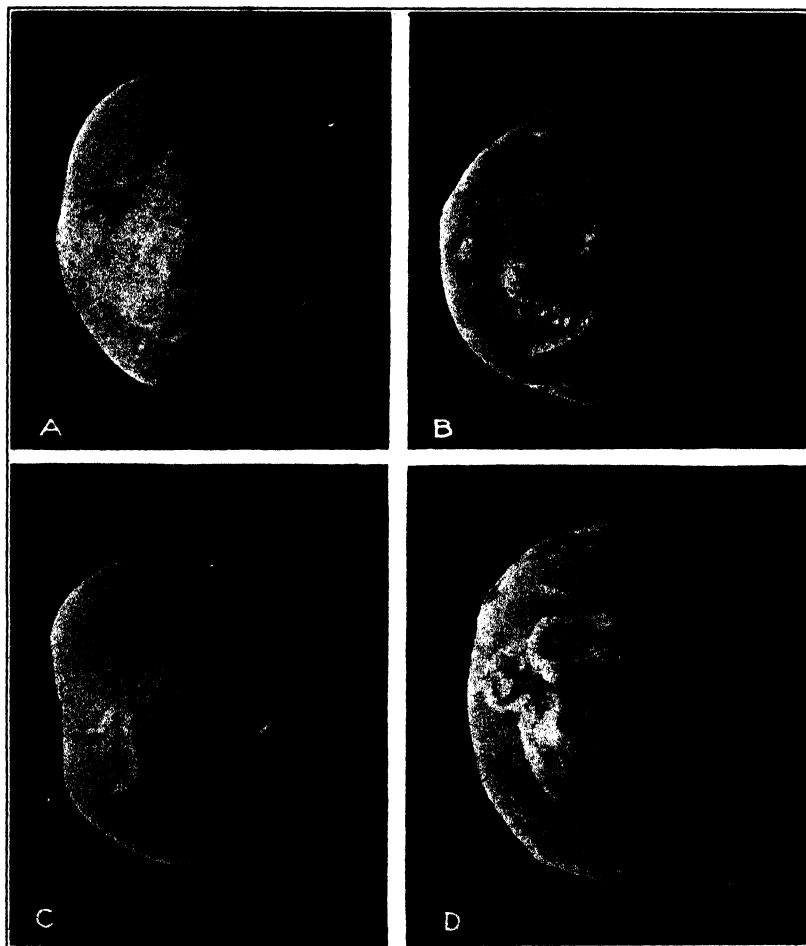


FIGURE 2.—Tubers grown from treated seeds: A, Showing inconspicuous flea-beetle injury; B-D, showing slight pimple and worm-track injury. A seeds were treated with organic mercury A; B, with organic mercury B; C, with organic mercury C; and D, with mercuric chloride

recurring rains made complete experimental management of the field impossible and interrupted the irrigation program.

Throughout the growing season the flea beetles were active and moderately abundant. The infestation was uniform. The foliage injury, as frequent investigation showed, was uniform over treated and untreated lots. The repellent spray was needed early in the growing season. The beetles were to be found after the above-mentioned hail, however.

The potatoes were harvested on September 26 and 27. In sorting, the immediately striking result was the variation in the amount of common scab (*Actinomyces scabies* (Thax.) Gues.) found in the tubers from seed potatoes receiving different treatments. Throughout the field the damaging amount of worm track occurred uniformly where the worst scab appeared. Representative samples from each treatment were reserved for laboratory study.

LABORATORY RESULTS

Of the bushel or more of potatoes saved from each treatment, each of which was a composite sample, all were examined for insect injury. There was some variation in severity in each of the seven lots. From each were selected several tubers characteristic of the lot. They were neither the cleanest nor those with the worst worm tracks. They were entirely average, characteristic, and representative.

The more severely affected tubers were marred by scars, which were often winding and crossing, usually raised, bulging, eruptive, sometimes ravelike, and discolored in the depths. In addition there were pimplelike swellings that seemed to be bursting from within. The tuber surfaces were rough, eczematous, and decidedly objectionable. The tubers showing the best appearance were smooth. The worm-track scars, though winding like the others, were smooth and only slightly different in color from the normal epidermis. The points at which the larvae had burrowed showed slight scars but no pimples. The older tracks were shallow and narrow, the normal epidermis developing a light feathery edge along the margin. Occasionally there would be a rougher spot, small in extent. The number of worm tracks and the burrows or tunnels were no fewer in the lightly infected than in the more severely infected tubers, but the appearance was greatly improved. The tubers in Figures 1 and 2 are typical of the lots represented. Each shows approximately the same degree of injury from attacks of the larvae, but with very considerable differences in the development of scars.

Tubers which were not attacked by flea-beetle larvae but which bore scab lesions showed characteristic symptoms of scab. The scab spots, which were usually flat, were much larger than a pimple injury, and the interiors were filled with soft corky tissue. True scab injury would not be confused with that caused by the scab organism invading larvae injuries.

CULTURE OF TUBERS

Tubers from each lot were cultured, pieces of tissue being taken from the worm-track areas and pimples. The method described by Shapovalov (12) was used. After the surface had been sterilized with hydrogen peroxide, the material was carried to sterile water blanks, and from these to melted nutrient agar, and poured into Petri dishes. In the case of the cleaner tubers, where the worm-track corky tissue was too shallow to be separated, the worm-track tissue was macerated and cultured. The dishes were incubated at 32° C. for 24 hours, examined, and isolations made. At a later time the growths were identified. The result of the culture work is shown in Table 1.

TABLE 1.—*Results of culturing representative worm-track and pimple injuries on tubers grown from treated and untreated seed*

Treatment of seed	Tubers cultured	Tubers yielding Actinomyces scabies		Tubers yielding Corticum vagum	
		Number	Per cent	Number	Per cent
Untreated:					
Cut.....	32	26	81.25	8	25.00
Whole.....	32	22	68.75	3	9.37
Graphite.....	20	18	90.00	3	15.00
Total or average.....	84	66	78.57	14	16.66
Treated:					
Organic mercury A.....	29	5	17.24	1	3.44
Organic mercury B.....	30	5	16.66	0	0
Organic mercury C.....	23	3	13.04	0	0
Mercuric chloride.....	27	5	18.51	0	0
Total or average.....	109	18	16.51	1	.92

DISCUSSION

The tubers of the untreated group show 78.57 per cent scab infection in the worm-track areas as compared with 16.51 per cent infection in the treated group. These results are not strictly comparable, however. In the untreated group the worm-track areas were heavily scabbed; the surface corky tissue was lifted off, and the tissue to be cultured was taken from beneath. In the treated group a thick corky tissue had not developed, and the worm-track areas were macerated and cultured. In examinations of the plates from the treated group the scab organism was commonly found growing from the epidermal surface and not from the corky tissue beneath. It appeared to be a surface-contaminating organism and not an infecting one. While the percentage (16.66) of *Rhizoctonia* found in the untreated group is moderate, the damage resulting from it was small. The worm tracks were damaged mostly by scab.

TABLE 2.—*Total and nonmarketable (scabbed) yields of potatoes, graded to marketable size, grown from treated and untreated seed*

Treatment of seed	Units ¹	Total weight		Scabbed potatoes	
		Number	Pounds	Weight	Per cent
Untreated:					
Cut.....	27	7,397	965	13.04	
Whole.....	9	2,386	302	12.65	
Graphite.....	9	2,288	249	10.88	
Total or average.....	45	12,071	1,516	12.56	
Treated:					
Organic mercury A.....	9	2,450	40	1.63	
Organic mercury B.....	9	2,535	38	1.49	
Organic mercury C.....	9	2,168	40	1.84	
Mercuric chloride.....	9	2,517	63	2.50	
Total or average.....	36	9,670	181	1.87	

¹ Each unit consisted of the total yield of a portion of row 300 feet long.

The weight and the percentage of scabbed potatoes found in the experimental field are shown in Table 2. The potatoes were sorted

for marketable size, and those that were nonsalable because of scab were taken out. This classification did not necessitate the elimination of tubers damaged by clean or scab-infested worm track. Table 2 shows that of the tubers grown from untreated seed 12.56 per cent (an unusually high percentage for the Greeley district) were unmarketable because of scab. Of the tubers grown from treated seed only 1.87 per cent were scabbed. It appears therefore, that the treatments, besides reducing infection of worm tracks, decrease scab.

The infection of the worm-track areas of the tuber by *Actinomyces scabies* and by the *Rhizoctonia* fungus (*Corticium vagum* Berk. and Curt.) has been observed for some time. Figure 3 shows a tuber of the Pearl variety deeply corroded by *Rhizoctonia* following worm-track injury. In the Greeley area scab and *Rhizoctonia* are usually moderate parasites, requiring suitable weather conditions for their best development. In 1920, at the place where the tuber shown in Figure 3 was taken, *Rhizoctonia* was well developed on all tubers where

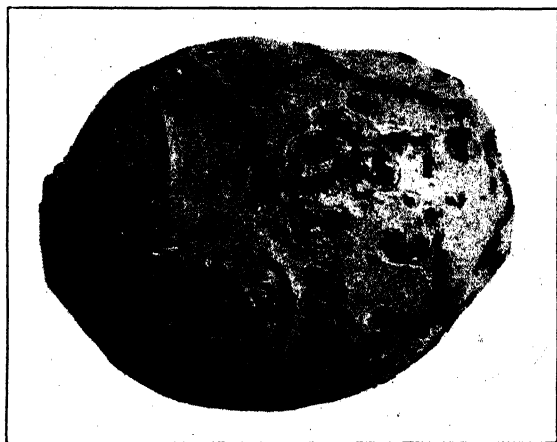


FIGURE 3.—Pearl tuber grown from untreated seed; badly damaged by *Rhizoctonia* which followed flea-beetle injury

injuries to the skin rendered infection easy. Scab likewise attacks tubers where the epidermis is thin, for thickness of the epidermis is one of the essential natural scab-resisting features of the potato. Jones (4) early noted that Cambridge Russet was scab resistant. Lutman (7, p. 30) found that the thickness of the skin determines the resistance of the tubers to scab. The effect of the feeding of the larvae on the tuber surface is essentially to

reduce the thickness of the periderm. The larvae eat away the epidermis and a few layers of cork cells beneath. More cork cells are produced from the phellogen to repair the damage, but a thin place remains in the protective covering of the tuber. Figure 4 shows a free-hand section through an uninfected worm track. At *a* is shown the normal epidermal tissue, consisting of the thickened epidermis, beneath which are the compact cork cells, and at *b* is the worm track itself, without epidermis but showing the growing cork cells. It is in this tissue that infection occurs. Lutman and Cunningham (8) found that the loose cellular formation of the tuber lenticel normally offered easy entrance to the scab organism. Lutman (6) had previously found that scab frequently occurred at lenticels. Recently Sanford (11) stated that lenticels are the principal points of scab infection. The injury caused by the larvae of the flea beetle creates a similar point of entry for the scab organism.

The conditions that favor the development of scab in the Greeley area differ from those generally reported as favoring scab develop-

ment. Sanford (10) found that abundant scab developed in very dry soils, while soils sufficiently moist produced almost clean tubers. Millard (9), commenting on the occurrence of scab in England, stated that it is more prevalent in dry than in wet seasons. Other investigators have contributed similar evidence. In the Greeley area the greatest scab development is during wet years, or on wet ground. The growing season of 1928 was unusually wet. Between June 15 and August 1, 6.53 inches of rain fell at the experimental field, or over one-half the average annual precipitation. The amount of scab aside from worm track was much larger than usual. In dry years scab is commonly found only at the lower ends of rows where irrigation water remains longest and drainage is poorest. Hoerner

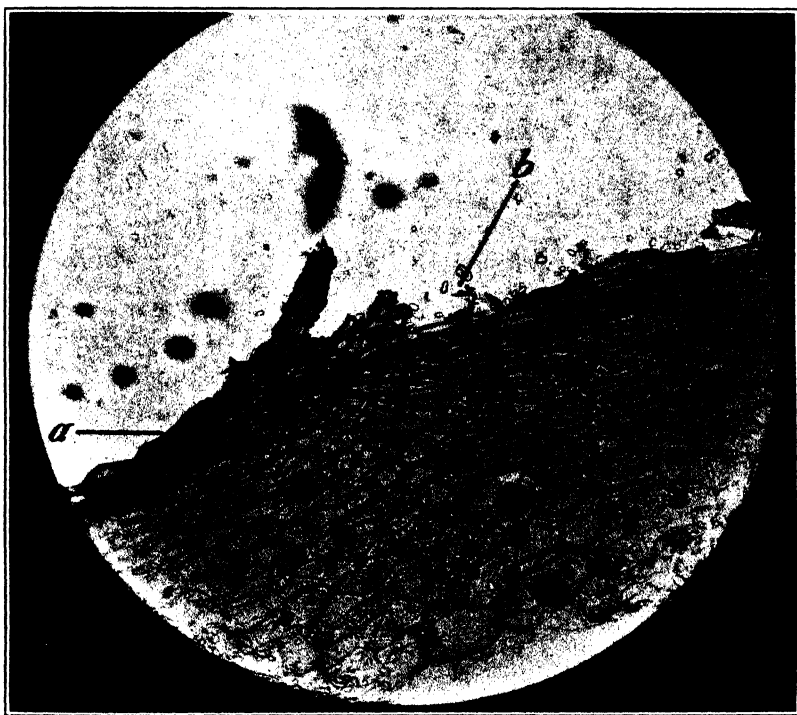


FIGURE 4.—Photomicrograph of a free-hand section through an uninfected worm track, showing normal epidermis and cork cells at *a*, and a portion of epidermis fed upon by a larva that caused the worm-track scar at *b*. This is without epidermis and shows loose cork cells formed from the phellogen

and Gillette (2) found that the severe worm-track injury occurred in wet ground or at the lower ends of irrigated rows, and also that smooth-skinned potatoes showed the greatest injury. The writers have made similar observations in regard to scab development in the Greeley area.

A suitable seed treatment effectively controls common scab under the conditions prevailing at Greeley. By eliminating the possibility of infection of the worm-tracked or tunneled areas, the most obvious damage to the tuber may be greatly reduced. The worm-tracked areas are relatively inconspicuous, and the points at which the larvae

tunnel do not develop the pimple type of injury. The question may well be asked whether the pimples described by entomologists (1, 3, 13) were not infected by scab. Where *Rhizoctonia* has been the active parasite following worm track the injuries are much more severe. Suitable treatment of seed potatoes for the control of scab and *Rhizoctonia* is an essential factor in the reduction of the tuber injury caused by the larvae of the flea beetle.

SUMMARY

The flea beetle (*Epitrix cucumeris*) has long damaged potatoes in the Greeley area of Colorado. The foliage is eaten by the adult beetles, and the damage caused by the larvae when feeding on the growing tubers has been a direct cause of economic loss. No complete control of the beetle or its larva has been found.

The damage caused by the larvae is of two kinds. The tuber surface is marred by the so-called worm tracks, and the places at which the larvae burrow into the flesh develop pimples. In the tuber flesh beneath the pimples are cores or slivers, which are found when the tuber is pared. The feeding of the larvae on the tuber, either in the portion appearing as tracks or at the point of burrowing, makes thin spots in the periderm, favorable to the entrance and infection of the spot by the scab organism (*Actinomyces scabies*).

If the seed potatoes have been suitably treated with mercury compounds the scab organism usually does not infect the larvae-injured potatoes. The tubers from treated seed, while attacked by the larvae as severely as tubers grown from untreated seed, show relatively little surface eruption or pimples. They are clean, bright, and smooth.

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OBSERVATIONS ON TASSELS OF TEOSINTE MALFORMED BY *SCLEROSPORA*¹

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INTRODUCTION

One of the most striking and commonly reported effects on cereals and other gramineous hosts resulting from attack by *Sclerospora* is the malformation of the inflorescence. This feature usually has been emphasized as conspicuous by many investigators during the last 50 years, who described these diseases on an extensive list of hosts, both cultivated and wild, comprising representatives in all but one or two of the less important tribes of the Gramineae. Of the tribe Maydeae (Tripsaceae), maize (*Zea mays*) has been found in Java, India, Formosa, and the Philippines to be seriously attacked by five separate species of conidial *Sclerospora* (6, 35, 38, 45, 46, 54, 56),³ at times with some concomitant malformation of the inflorescence. Yet in the case of teosinte (*Euchlaena* spp.), although it is perhaps the closest relative of maize and (as might be expected therefore) also is susceptible to these downy mildews, no instances of deformation have been reported hitherto. In the Philippines, however, while studying the *Sclerosporas* chiefly destructive to maize but also attacking teosinte, the senior writer, during two years of investigation and among many teosinte plants relatively unaltered by such attack, did encounter certain cases of abnormalities developing as a result of inoculation with *Sclerospora philippinensis*. It is with the hope that their rarity, their remarkable structure, and their possible significance may render them of interest that these cases are described in the present paper.

HISTORY OF THE CASES

The first planting of teosinte at the College of Agriculture of the Philippines was a small experimental plot started in July, 1917, with seed of *Euchlaena luxurians* Schrad. obtained from a New York seedsman. With seed from this a second planting was grown from June to November, 1918. In this second plot, and among the volunteer plants which sprang up around the maize then planted in the first, many instances of infection by *Sclerospora philippinensis* were noticed, but only a few were at all deformed and these but slightly (54, p. 104). With healthy seed from this plot seedlings were grown in pots protected in the laboratory. Beginning January 25, 1919, when they were 9 days old, with two leaves and about 3 inches high, the seedlings were placed for seven successive nights under young

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² To G. N. Collins for his kindness in critically reading this manuscript the writers wish to acknowledge their indebtedness; also, to the Milton Research Fund of Harvard University the senior writer wishes to acknowledge his gratitude for aid which, by lessening some of the demands of class work, has expedited the long-delayed preparation of parts of this paper.

³ Reference is made by number (italic) to "Literature cited," p. 833.

maize plants infected with *S. philippinensis* and showing abundant nocturnal production of conidia. To prevent extraneous infection the seedlings were covered each night with large cans. After being thus subjected to certain infection, they were allowed to develop in the laboratory until, on February 6, several of them (now 5 inches high and with the third leaf developing) showed the first paling leaf areas that precede production of conidia (57). Sixty of these seedlings were then planted in a corner of an isolated abaca and tobacco patch far from any other cases of downy mildew, and the progress of the disease on individual plants and in the plot as a whole was followed.

By the end of March many plants had died, under these crowded conditions, leaving only nine living, of which three were still healthy and remained so. In late April and early May, 1919, when conidiophore production on the six diseased plants had ceased save on young leaves of shoots recently put out, the male inflorescences (tassels) both on the main axes and on side shoots were found to be developing into abnormal, malformed structures, in contrast to the normal ones of the three still healthy survivors. These tassels, instead of producing pollen and becoming passé, remained green, the deformed glumes and lemmas of the hypertrophied spikelets continuing to grow excessively into contorted, elongate, bractlike structures which gave them a most bizarre appearance. A representative specimen was photographed (fig. 1) and preserved in copper acetate solution for future study; the others were kept under further observation.

During June a few additional tassels, also malformed, continued to develop from the later suckers of the diseased plants. This isolated plot was not visited during July, as in the latter part of that month there began a series of successive and severe typhoons that raged as one intermittent storm for six weeks. When visited on August 6 the plants were found to have been blown over and beaten down into the mud by the violence of the storms. The plants themselves had responded indomitably to this severe treatment by taking root and sending up new shoots at each node, but of the remarkable malformed tassels only a few survived, the several others, already old, having been beaten and soaked to pieces. These few survivors were collected, and from two of them were taken the peculiar rooting plantlets which, as herein described, were planted in the endeavor to foster their independent growth. As these malformed tassels seemed to present features of unusual interest, all possibly pertinent information concerning them was recorded, various illustrative photographs were taken, and the specimens themselves were preserved in formalin and in alcohol for more thorough examination later.

BEHAVIOR OF THE MALFORMED TASSELS

When the few plants whose malformed tassels had survived the severe storms were collected on August 6 it was with the intention of making a complete record of each one by means of photographs and notes, and this was carried out in part. The storms, however, became so violent that they prevented the completion of the work, and two remaining plants were left in the shelter of a tree on the lawn. These showed remarkable behavior, for when they were examined on August 13, during a lull in the storm, the upper portions with only slightly

deformed spikelets were found to have rotted apart and disintegrated, while the strikingly malformed spikelets of the more basal portion of the tassels had remained green and sound. Indeed, several of the



FIGURE 1.—Malformed tassel of teosinte: A, Upper portion of a plant of teosinte infected by *Sclerospora philippinensis*, showing the malformed tassel in position. This plant was one of a lot sprouted from seed planted in pots on January 17, 1919, inoculated as young seedlings with conidia of *S. philippinensis* from maize during the nights of January 25 to 31, planted on February 6 in an isolated plot, where, supporting the disease with little apparent effect beyond the striping of conidiophore-bearing areas of the leaves, the excessive production of tillers, and the suppression of the female inflorescence, the plants in late April developed the tassels. In June, when this specimen was photographed, the infected tassels were still flourishing vigorously, in marked contrast to the already passé tassels of comparable healthy plants. B, The same tassel more highly magnified to show its structure in greater detail. It consists of two long, approximately equal branches bearing spikelets, all of which are sterile, but most of them superficially relatively unchanged, although a few, as the one spikelet at the base of the right-hand branch and the six spikelets in the lower half of the left-hand branch, show malformation into striking, contorted outgrowths involving especially hypertrophy of the glumes and lemmas. The illustration is from two smaller photographs pasted together. About three-quarters natural size.

more elaborately hypertrophied spikelets, resembling deformed seedling plantlets, had actually sent out roots from their bases (fig. 2, A, B, C, D) as though they were capable of independent existence; whereas

some, as shown in Figure 2, A, *a*, *c*, and *d*, had practically separated from the prostrate tassel and had changed their orientation with relation to it until they had almost assumed the upright position which would be natural to young seedling plants of their size. Several of these, including those illustrated in Figure 2, B and D, were planted in a pot. Although they remained vigorous and continued to grow for more than a week longer, they were unable (probably in part because in the continuing storms they had almost no sunlight and were constantly far too wet and exposed) to establish themselves as independent plants and ultimately became water-soaked and rotten.

The development of the branchlets of these deformed tassels into entities comprising leafy shoots, giving rise at their bases to roots, orienting themselves like young plants, and for a time continuing to grow when quite separated from the tassel of which they had been a part, seems to justify regarding these as cases of apogamy. This holds, of course, only if the term is used in its general application, that is, to instances in which new plants develop nonsexually from parts that normally produce sexual organs, and not if the term is used in its more restricted cytological application. The behavior of these specimens, therefore, is of some interest, for in the Gramineae cases of apogamy in general are rare, whereas cases resulting from the attack of parasitic fungi have not been reported, as far as the writers are aware. As might perhaps be expected from the close relationship of the two genera, the examples of apogamy in maize (healthy, not parasitized) described by Collins (9) show striking resemblance in structure to those of teosinte now under consideration, although it should be noted that the plantlets which developed in the maize tassels terminated in small female inflorescences, whereas those of teosinte, if not completely sterile, showed only male rudiments. Moreover, the plantlets from the maize tassels when transplanted by Collins, even though they did not reach maturity, grew in an apparently normal way for nearly two months, producing roots more than a foot in length, thus far exceeding the amount or extent of the continued growth of the teosinte plantlets. The growth of these teosinte plantlets, however, even though scanty, was enough to show certain interesting aspects, for their development apparently resulted from attack by the *Sclerospora*, or at least followed it, and if they had lived they would have transmitted a novel type of primary infection to the plants into which they matured, since as long as the plantlets persisted they harbored within their tissue mycelium that remained living and apparently quite capable of renewed growth.

GENERAL STRUCTURE OF THE TASSELS

The appearance of the tassels as a whole was more or less striking, depending on the degree of malformation, but in all cases it was enough to arrest attention. In contrast to the normal graceful tassel with its several slender digitately diverging branches set with regular rows of small, smoothly fusiform, tapered spikelets of appressed florets, the inflorescences were frequently telescoped with reduced branching to bunchy, tufted heads, or rendered unsymmetrical and bizarre in contour by flaring, uncouth, sprawling outgrowths.

In the bunchy type, representing one extreme of the forms encountered, the branching was suppressed, the whole inflorescence being

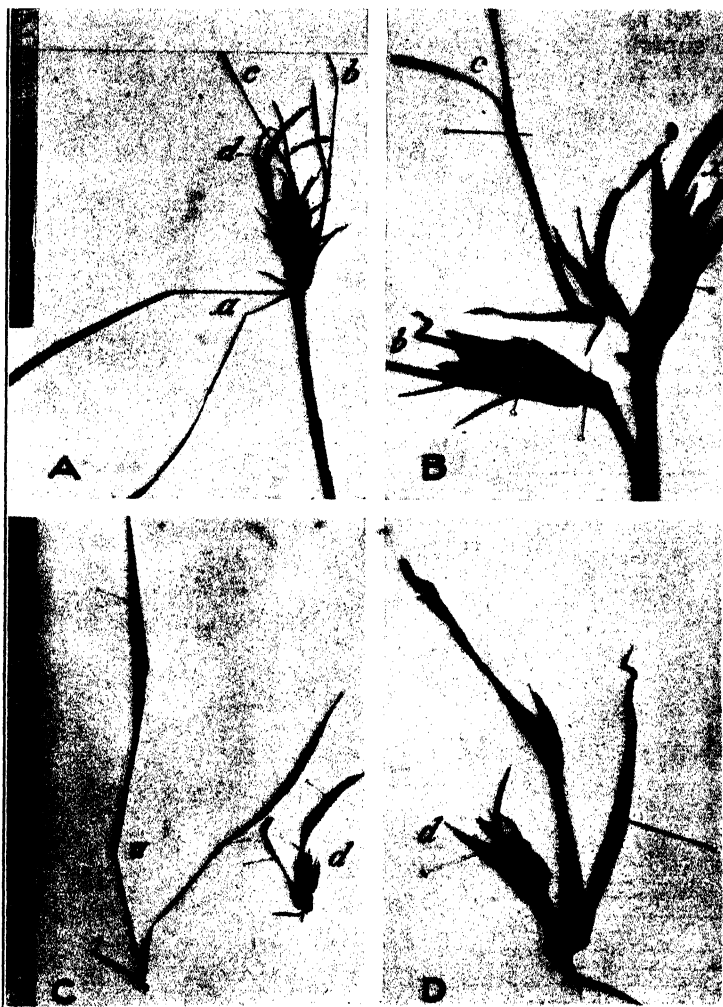


FIGURE 2.—Malformation and root development of parts of teosinte tassel: A, The basal portion of a male inflorescence of teosinte taken from a plant of the same lot and with the same history as the one illustrated in Figure 1. This plant, however, after surviving a long period of incessant storms until August 6, was pulled up and then left lying during an especially violent typhoon until August 13, when, although the top of the tassel had rotted apart and the less malformed spikelets of that portion had begun to disintegrate, the more elaborately malformed spikelets at the base of the inflorescence had continued growing and had sprouted roots at their bases. Scale in inches. B, The base of the same tassel with some of the seedlinglike branchlets removed and the remaining parts separated to show the general structure. The branchlet *c*, previously obscured by the lower clump of spikelets *b* now pinned down away from it, shows a general resemblance to a seedling plant with its base, almost separated from the tassel axis, having one short but obvious root growing down from it. The broken end of the main axis, from which the top of the tassel disarticulated, is shown at *x*. About two-thirds natural size. C, Two of the deformed parts, *a* and *d*, removed from the tassel shown in A. The slender one, *a*, is recognizable as a single spikelet with glumes and lemmas so overgrown into elongate leaflike structures (6 and 8 inches long) that the whole entity with its few short, poorly developed roots at the base resembles a sickly seedling plant of teosinte. The shorter one, *d*, is a compact clump comprising at least two spikelets, much malformed, rooting at the base. Scale in inches. D, The malformed branchlet, *d*, already shown in C after removal from its place (*d*) in the tassel of A, spread apart and photographed somewhat larger to show the general structural features of the several parts and the growth of the roots at their base. About two-thirds natural size.

shortened and condensed into a single compact, clumped, bunchy head, superficially resembling the inflorescence of *Setaria* or *Pennisetum*, as is shown in Figure 2, A. Even the more open branching type, less completely deformed, less departing from the normal, and representing the other extreme encountered, showed more or less numerous striking, elaborate, twisted, curved outgrowths that, as shown in Figure 1, at once marked them as abnormal. In their gross structure these male inflorescences presented a striking contrast to those healthy ones illustrated and described by Collins (14, 16), by Weatherwax (53), and by others. While more detailed morphology and histology of these malformations will be considered later, it is worthy of note that in general the spikelets of the tassel, especially the more basal ones (fig. 1), were strikingly hypertrophied, the lemmas particularly enlarging astonishingly into expanded leaflike structures (fig. 2), and the whole spikelet elaborated until in some cases it much resembled a somewhat deformed seedling plantlet (fig. 2, B, C, D) sprouting from the joints of the tassel branches. Moreover, the stamens were lacking entirely or else abortively developed, so that all the spikelets of the tassels were sterile. This was true not only in the badly deformed, more basal spikelets, as might be expected, but also in those usually nearer the tip, which appeared superficially unaltered.

In view of the elaborate development of these deformed spikelets, it is remarkable that all the cases showed no malformation except in the tassels. The plants themselves were not seriously injured and, like most of the *Sclerospora*-infected teosinte individuals already studied, described, and illustrated by the senior writer (57, *pl. 6*; 54, *pl. 22c*), had shown during the period of conidiophore production relatively inconspicuous striping and stiffening of the leaves, had continued growing unimpeded, and at maturity, when production had ceased, were only faintly mottled, although the mycelium was still present in the leaf tissue. Moreover, these plants without exception did not develop any female inflorescence, thus lacking opportunity for the malformations that frequently occur in those structures in downy-mildewed maize plants.

DETAILED STRUCTURE OF THE TASSELS

The representative specimens which had been preserved in the Philippines eventually were brought to Harvard University, where a detailed study of the floral structure and the histology of the tassels was made by the junior writer. The floral parts of these malformed specimens were carefully unrolled, dissected, drawn under the camera lucida, and compared with one another and with healthy material. Also, critical structures and parts were embedded in paraffin, cut in several directions in sections of various thicknesses, and stained with various combinations, such as Flemming's triple, Pianese 3, or haematoxylin and Orange G.

As the general symptoms of this disease in teosinte plants have been described in earlier papers by the senior writer (54, 57), attention was directed to the staminate inflorescence in which the malformations occurred.

The normal, healthy, staminate inflorescence of teosinte resembles that of Indian corn except that in the former the central spike is suppressed. The spikelets are 2-flowered and arise in pairs at each

joint of the rachis and on one side of it. One spikelet of each pair is subsessile, the other one is pediceled. These features are well known from the descriptions and illustrations of Collins (13, 16), Weatherwax (53), and others.

In the tassels of the diseased plants various gradations of transformation existed. As a rule, the sessile spikelets were more excessively malformed than were the pediceled ones, and those at the base of the spike more than those toward the tip. In some of the diseased plants the general appearance of the spikes was not greatly altered, but in other cases only a few joints of the rachis developed, and the spikelets here were generally contorted and excessively hypertrophied, so that they presented a "bunchy" appearance in which practically all semblance of a spike was lost. Each joint of the rachis, however, almost invariably bore its two spikelets. Sometimes both of these were pedicellate.

One case different from any of the others was observed. Two spikelets were raised on a short pedicel and inclosed by three glumes. (Fig. 3, H.) One of these glumes was somewhat larger than either of the other two, but there were no indications that it resulted from a fusion of two. That these spikelets were indeed spikelets and not florets is shown by the fact that each one possessed two florets whose composition and arrangement were identical with those of other diseased spikelets.

Gabotto (19) relates that in a study of maize infected with *Sclerospora macrospora* he counted 60 virescent staminate inflorescences of abnormal development and states that, since the plants did not produce ears, the parasite seemed to stimulate excessive growth of some parts at the expense of others. A somewhat similar effect was evident in this material. In those branches which retained their spikelike formation the excessive overgrowth of the spikelets on the lower portion of the rachis seemed to take place at the expense of spikelets higher up. Indeed, from the base to the tip of the rachis there was manifest a tendency toward the progressive reduction, and finally the suppression, of the several parts of the florets. The second floret of each spikelet was invariably smaller than the first floret and first suffered the loss of one or more of its floral elements. At a distance up the rachis of approximately one-third the entire length it lost its palea. Slightly higher a similar loss was sustained by the sessile floret. The lemmas of each of the florets were still present at that height, but about halfway up the rachis the lemma of the second floret usually disappeared, and somewhat farther up the first floret lost its lemma, so that in the topmost spikelets, more often than not, only the two glumes were present. (Fig. 3, F.) Occasionally the first glumes alone remained. As the spikes of diseased tassels differed much in length and were always shorter than those of healthy tassels, it can readily be understood that in extreme cases the upper portion of the spikes would fail to develop at all and the bunchy type of tassel already mentioned would result. In the more detailed account of the floral structure which follows, the features referred to will apply particularly to excessively hypertrophied spikelets, the applicability of the description diminishing proportionately as the spikelets approached normal development.

The glumes shared in the general overdevelopment of the spikelet, but rarely lost their glumelike character. Their increase in width and thickness was proportionate to their increased length, which in many

cases exceeded three times that of the normal glume. (Fig. 3, A, B, H.) More vascular bundles were present than in the healthy glumes,

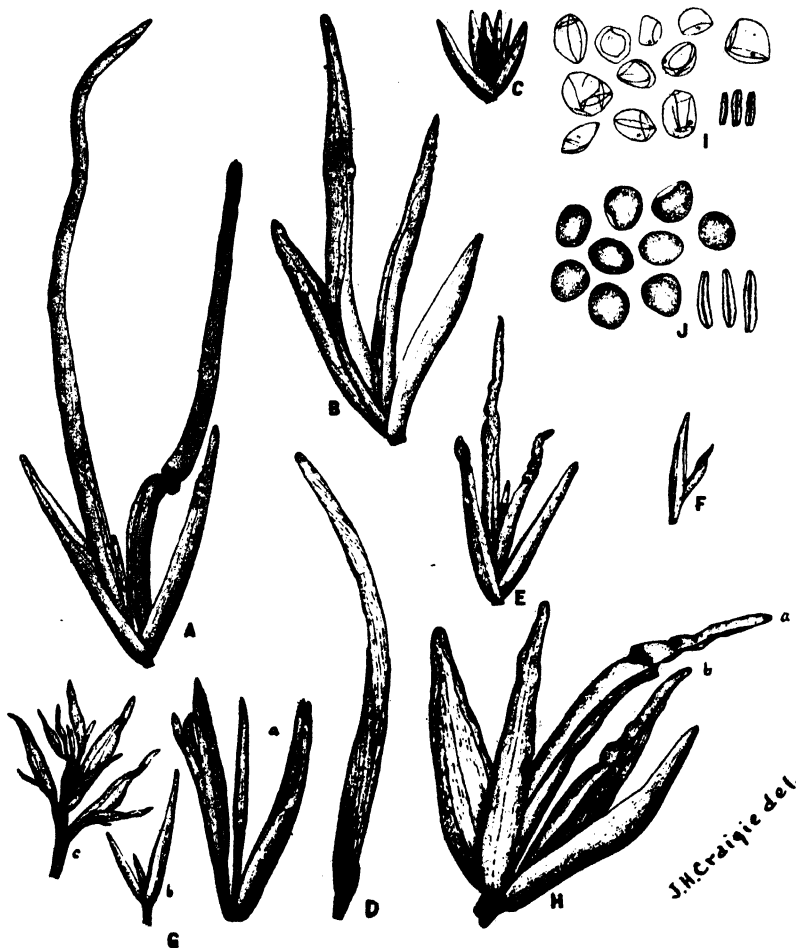


FIGURE 3.—Structure of malformed spikelets from tassels of infected teosinte: A, B, E., Staminate spikelets of teosinte hypertrophied and malformed by an attack of *Sclerospora philippinensis*. The tightly enwrapped floral elements were opened and spread apart to show the position and relative size of each. In A the lemma of the second floret had to be twisted from its original position in order to expose the palea. The portion above the knotlike formation was not enveloped by the other lemma. $\times 1\frac{1}{2}$. C, Spikelet of healthy tassel, showing stamens in first floret, and affording an interesting comparison in size, proportions, and construction to those of the diseased. $\times 1\frac{1}{2}$. D, Spikelet of diseased tassel showing hornlike form characteristic of many diseased spikelets. $\times 1\frac{1}{2}$. F, Spikelet from tip of a diseased tassel, floral parts reduced to two. $\times 1\frac{1}{2}$. G, a, Three spikelets which arose at one joint of a rachis; b, central spikelet opened, consisting of three floral parts; c, proliferation of florets found within the glumes of the left spikelet of a. $\times 1\frac{1}{2}$. H, Two spikelets, a and b, growing on a common short pedicel and inclosed by three glumes. $\times 2$. I, Pollen grains and anthers from diseased florets. The grains are shrunken and collapsed, with variously wrinkled and infolded walls and with content almost devoid of protoplasm, so that the germ pores appear very distinct. The anthers from the diseased florets are small and poorly filled out. Pollen grains, $\times 78$; anthers, $\times 2\frac{1}{2}$. J, Healthy pollen grains and anthers, drawn to the same scales of magnification as I, in order that they may be compared with the pollen grains and anthers of the infected plants.

and as a consequence of this and the greater thickness the glumes were quite rigid. The hispidness characteristic of the glumes was much more pronounced in these than in the healthy ones.

The lemmas, on the other hand, underwent astonishing transformations and usually assumed a very extraordinary arrangement. Although in healthy florets the lemmas are membranaceous and hyaline, with any trace of hispidness visible only microscopically, several of the diseased ones were leaflike and virescent, while most of the others were distinctly hispid and rigid, resembling very much in texture the diseased glumes. These observations apply more particularly to the lemma of the first floret of each spikelet, for in most cases the arrangement of the floral parts seemed to forbid such extravagant development in the other parts as was permitted in this one. In practically every diseased spikelet examined, the lemma of the first floret tightly enwrapped its palea (and stamens when present) and the second floret either wholly or in part. In doing this it usually assumed a somewhat spiral course, so that when it completely enveloped the other floral elements it presented the appearance of a horn. (Fig. 3, D.) Occasionally this formation was quite regular, but more frequently it showed various degrees of torsion and wrinkling. (Fig. 1, B; fig. 3, E.) When the second floret was not completely encircled, the upper portion being sometimes uncovered, the exposed portion was very similar in appearance to the enwrapping lemma. (Fig. 3, A.)

In a few cases there were modifications of this general arrangement. The most interesting one of these was the differentiation in several cases of extremely enlarged lemmas into sheath and blade, as in a leaf. (Fig. 2, B.) The lower portion was tightly enrolled after the manner of a sheath, but the upper portion flattened out into a well-formed blade. (Fig. 2, A, B.) Just at the top of the sheath a ligule was almost invariably present. It never developed completely around, but extended inward from either edge for about one-third the width. The central portion of the ligule failed to develop. In the cases just mentioned the upper portion of the lemma of the second florets was left exposed, and two or three of them expanded into virescent blades, but no ligule formation was observed.

Toumey (50), in describing abnormalities in the inflorescence of *Phleum pratense*, mentions that some of the flowering glumes were changed into leaves. Butler (5) did not observe any transformation of this kind in *Pennisetum typhoideum* attacked by *Sclerospora graminicola*, although other abnormalities were produced. He states, however, that the lemmas were usually elongated, occasionally virescent, and softer than healthy ones.

Although each joint of the rachis regularly bore two spikelets, a case was observed in which three spikelets arose from the same joint (fig. 3, G, a), one of which was subsessile, the other two pedicellate. Of these last two, one was situated centrally and possessed but one floret (b), the other resembled in external characters the ordinary diseased spikelets, but when its glumes were removed it disclosed a very unique construction. The rachilla, which in other spikelets bore two florets, was elongated considerably and bore seven spikelets. (Fig. 3, G, c.) These were not arranged in quite the orderly manner that was evident in the ordinary spike. The first spikelet on this elongated rachilla was sessile, the next one above it pediceled. The fourth was raised on a comparatively long pedicel in the axis of the third, which was sessile. The three uppermost arose separately and were greatly reduced, two of them being represented by only a single glume each, the third one by two very

diminutive glumelike structures. In this particular case the rachilla had evidently assumed the function of a rachis and bore spikelets instead of florets. The term "spikelet" is applied to these structures for the reason that the glumelike character of the outer element of each was very evident, and one spikelet consisted of three bracts, whereas, if it were a floret, it would have had but two—the lemma and the palea.

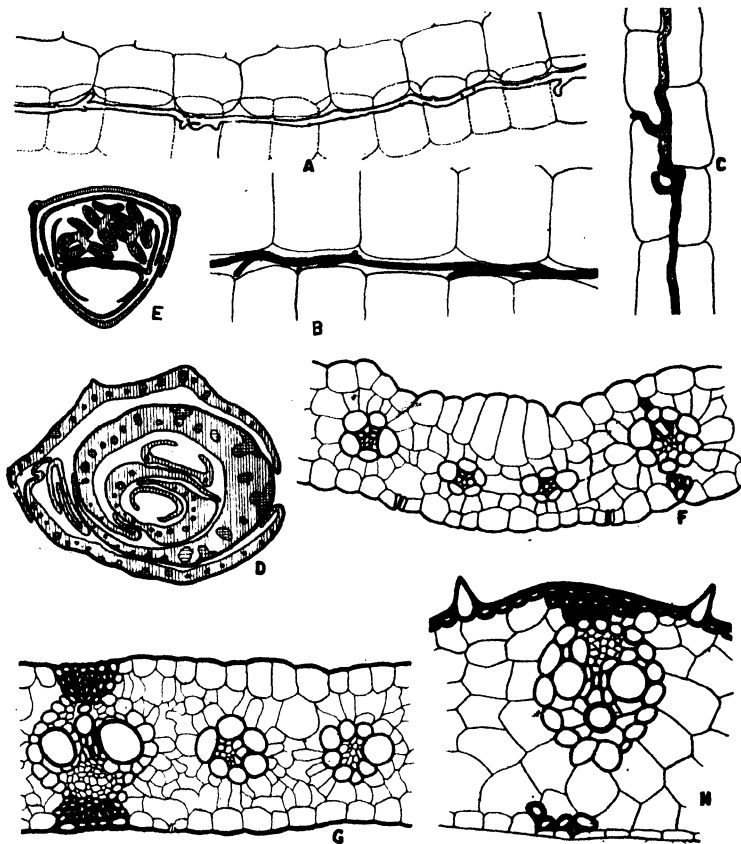
This aberrancy would seem to fall in teratological classification under "median proliferation." Moquin-Tandon (37) speaks of proliferation as "median" when an adventitious bud springs from the center of the flower as a direct continuation of the growing point. In describing proliferation in flowers, Masters (32) states that in median proliferation the adventitious bud springs from the center of the flower; the usual arrest of growth which occurs at this spot no longer holds good, but a new growth takes place, manifesting itself generally in the formation of a new flower bud, a new leaf bud, a branch, or even in the production of an inflorescence. Toumey (50) described a case in which some timothy spikelets had four glumes, some only two, and between the inner ones a long stipe, generally more or less twisted, arose and was surmounted by a perfect flower. This irregularity in structure Penzig (44) termed "central proliferation."

The dissimilarities of the internal structure of healthy and diseased lemmas were not less marked than those of their outward form. Healthy lemmas, for the most part, were of a uniform thickness, mainly one cell thick, and therefore contained no mesophyll or well-defined vascular bundles. Diseased ones, on the other hand, were of uneven thickness, having well-developed mesophyll and fairly regularly spaced vascular bundles (fig. 4, F, H), some of which were quite perfect, with vessels, phloem, and sheath, while others perhaps, consisted of only two or three vessels, or presented gradations between these two extremes.

As the morphology of those portions which flattened out to form blades was considerably different from that of the rest, it will be mentioned first. A comparison of the two cross sections F and G of Figure 4 shows how closely the former approximated the regular leaf formation of the latter. In both cases the upper and lower external walls of the epidermis were of about the same thickness, although in the leaf, G, these were slightly thicker than in the blade-like lemma, F. Also, the leaf itself was somewhat thicker. The vascular bundles in the bladelike lemma were regularly spaced and were as complete as many of those in the leaf, yet at intervals the leaf possessed bundles which, above and below, were abutted by a group of thick-walled sclerenchymatous cells which extended to the epidermis. These were absent in the bladelike lemmas, but in general their structure corresponded very closely to that of the leaf.

The inrolled lemmas, on the other hand, differed quite materially from the flattened portions or from a leaf. As seen in cross section, their margins were thin, but a progressive thickening took place toward the middle, so that in this region there was a spongy mesophyll, as in the leaf. (Fig. 4, D.) There were present also more or less regularly spaced vascular bundles. Toward the edges, and therefore in the thinner portion, these were always incomplete, being composed usually of a few vessels. In the thicker portions some were quite complete (fig. 4, H) and others were imperfect like

those in the thinner parts. The cell walls of the outer epidermis were thick and sclerenchymatous, and they frequently protruded, so that this surface was studded with short stout hairs. Stomata



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FIGURE 4.—Details of internal structure of malformed spikelets: A, Thick-walled strand of mycelium without cytoplasmic content, showing swellings and prominences which press against the cell walls. $\times 200$. B, Thick-walled strand of mycelium with content. The host cells have been wedged apart by the mycelium. $\times 200$. C, Portion of thin-walled hyphal strand with cytoplasmic content. $\times 200$. D, Schematic representation of a cross section of a deformed spikelet, cut 3 mm. above the base, to show the inrolled nature of the lemmas. In this spikelet an additional abnormality appears in the form of an extra floral bract just inside the glumes. The vascular bundles and sclerenchymatous tissue are crosshatched. \times about 14. E, Schematic representation of a cross section of a healthy spikelet. \times about 14. F, Portion of a cross section of a virescent lemma, showing its similarity in internal structure to the normal leaf. $\times 200$. G, Portion of a cross section of a healthy leaf. $\times 200$. H, Cross section of a portion of strongly inrolled lemma at its thickest part, showing the external thick-walled epidermis, the sclerenchymatous reinforcement cells which connect with the bundle, and the thin-walled inner epidermis with its adjoining small group of thick-walled cells. $\times 200$

occurred sparsely. Abutting on the epidermal cells and just over the bundles were one or more layers of thick-walled cells. Where the bundles were large and more complete, these cells bridged the space between the epidermis and the bundle sheath (fig. 4, H), but

where the bundles were more or less imperfect, thin-walled cells usually intervened between these thick-walled cells and the bundle. On the opposite side of these bundles and lying adjacent to the inner epidermis there were usually a few thick-walled cells, but these never connected with the bundles. (Fig. 4, H.) The walls of the inner epidermis were thin, like those of the mesophyll cells, and few stomata or hairs were evident on this side. This condition—a firm outer epidermal layer, reinforced by clusters of thick-walled cells and opposed on the inside by only a thin-walled epidermis—seems to account satisfactorily for the inrolling that took place in so many of the diseased lemmas.

As already stated, the lemma of the second floret of the spikelet was either partly or wholly enveloped by that of the first floret, and as an apparent consequence (although it duplicated in a general way the features already indicated of the enwrapping lemma) these features were not so pronounced. The thick-walled outer epidermis, the strengthening clusters of fibers, and the vascular bundles were present, but less conspicuously developed. However, in those cases where its upper portion became uncovered and thus exposed to sunlight, it took on the characters of the enwrapping lemma, which seemed to indicate that if this lemma had had an exposure to sun and air it would have been identical with the one that inclosed it. The leaflike appearance of two or three of these has been mentioned.

The palea of the first floret participated somewhat in the general hyperplastic condition and became frequently three times its normal size, but in no case did it approach the extravagant dimensions of the corresponding lemma. In the second floret, the palea, surrounded as it was by two lemmas, was apparently stifled and only rarely attained the size of the other palea. As a rule, it was poorly developed, often only a vestige being present, and it was more frequently absent than any of the other floral bracts.

The stamens, more often than not, were suppressed. If present, the anthers appeared atrophied and were rarely more than two-thirds their normal size. The pollen grains varied greatly in size and shape, some being even larger than the normal healthy grains, while others, again, were reduced to half that size. (Fig. 3, I, J.) All of the pollen grains in a diseased plant were practically devoid of content, so that the walls became wrinkled and infolded, and the appearance of the grain was irregular and bizarre. (Fig. 3, I.) None of the modifications that Butler (5) described for the stamens of *Pennisetum typhoideum* attacked by *Sclerospora graminicola* (Sacc.) Schroet. was observed.

As the characteristics of the mycelium of this fungus have been described by the senior writer in a previous publication (54), it is unnecessary to repeat them here. It might be added, however, that the hyphal filaments that seem to serve for communication between different parts of the host were frequently, though not always, very thick walled, a condition resembling that observed by Butler (5) in *Sclerospora graminicola* on *Pennisetum*. Usually these filaments were devoid of cytoplasmic content. A portion of a thick-walled hyphal filament without content (A) and a portion of one with content (B) are shown in Figure 4, as well as a thin-walled portion of a filament (C).

DISCUSSION

These cases of malformation involve several points of interest. Not only are they remarkable because of their rarity in the Gramineae in general and in teosinte in particular, but also because of their elaborate structural overdevelopment in contrast to their complete sterility, their growth as independent plantlets, and their resulting abortive apogamy. Moreover, they present an interesting comparison to cases of malformations in other grasses and contribute to possible interpretations of such structures and their significance.

In the first place, these malformations are of interest because in teosinte the occurrence of structural abnormalities of any kind, whether resulting from injury by downy mildew or from any other factor, is exceedingly unusual. Although numerous plantings of teosinte from several lots of seed obtained through the kindness of G. N. Collins and others from Mexico, Florida, and other localities were kept under observation for two years in the Philippines, the senior writer found exceedingly few and slight abnormalities either in healthy or in downy-mildewed plants with the exception of the present cases. Moreover, Collins, who has studied extensive plantings for many years, summed up his long experience in a lecture in 1918 (12) with the statement:

Compared with maize, teosinte is absolutely constant. This is true whether looked at from the standpoint of fluctuating variations of a quantitative nature or the frequency of occurrence of abnormalities. We have had teosinte under observation every season for 10 years, and during that time, with the exception of some apogamous plants one season at San Diego, not a single pronounced abnormality has appeared.

Nine plants survived the crowded conditions in the senior writer's test plot in the Philippines. That the six infected ones of these all developed decidedly malformed tassels is therefore the more striking. It should be noted in this connection that from seed of representative plants in the parent plot Collins made certain that this teosinte was pure and free from contaminating hybridization with maize.

In the second place, these malformations are of interest in themselves because they show such remarkable modifications in structure. The elaborate overdevelopment of such floral parts as the glumes and lemmas; their hypertrophy to many times the normal extent and bulk; the formation of additional fibrovascular bundles within them; the marked alteration in size, position, arrangement, and relation of the florets and their parts; the local unevenness of growth with consequent crumpling and curling of mature organs; and the binding and restraint of rapidly expanding inner portions of the inflorescence by the adherent unexpanding outer parts enfolding them, with consequent contortion, buckling, and tearing—all these features mark these malformed inflorescences as noteworthy cases of abnormal growth. As such they may be of interest in the field of teratology, a branch of botany little emphasized at present, but one in which in the past Penzig (44), Masters (33), Moquin-Tandon (37), and others not only devised extensive classifications and terminologies, but also made comparative studies of the structural modifications in different groups of plants and worked out convergences and homologies that are still of some general significance. The extensive deformation of these teosinte tassels is of added interest because it seemingly was consequent to the irritation of the downy-mildew mycelium growing immediately within their tissues, an action which apparently

in most cases was direct, as in very few instances did the apparent absence of hyphae within the deformed floral parts permit consideration of possible action at a distance.

In the third place, these malformed inflorescences are of interest because they were completely sterile. This was true not only in cases where the spikelets entirely lacked sexual organs and were profoundly modified, but also in cases where the florets in general appearance were almost normal and developed stamens, for the pollen was invariably abortive and nonfunctional. Moreover, they were sterile with respect to both sexes, for in no case did female branches develop. This sterility, however, was accompanied by unusual vegetative rejuvenescence. Uncommonly numerous shoots were developed continuously from the bases of the plants, and when during the heavy storms the plants were beaten down into the mud, they sent up many new sprouts from their nodes. The continued proliferation was so pronounced that during the six months they were under observation the plants, although of the annual species of teosinte, in general resembled the perennial type that occurs in Mexico and has been studied and described by Collins (14). That these plants should be sterile florally and yet have such unusually prolific vegetative growth gave rise to the rather anomalous situation that if the plants had been grown for seed or to furnish pollen for hybridization the effect of the downy mildew would have been regarded justly as very injurious, quite as it is in the case of other grain-bearing crops such as maize or wheat; whereas, if the plants had been wanted for their vegetative growth for fodder or stover, the effect of the mildew on the whole was beneficial, rendering them even more valuable than the less prolific healthy individuals.

It is of interest also that coincident with the sterility of these plants there took place an abortive apogamous reproduction through the temporarily independent growth of the seedlinglike spikelets, for although examples of this viviparous method of propagation have been observed in the Gramineae they apparently are rare in the tribe Maydeae. The cases of apogamy in maize reported by Collins (9) are the only ones in the tribe described hitherto, and the cases in teosinte (unfortunately not described but merely mentioned in the same paper) are the only ones ever even recorded in that genus, as far as the writers have been able to ascertain.

In the fourth place, malformations such as these in teosinte may perhaps have some structural or phylogenetic significance as traumatic reversions to ancestral conditions. The consistently leaflike structure of the abnormal growths which develop from diverse injurious agents in the several Gramineae considered probably may be interpreted as evidence for the theory that the leaf is the ultimate progenitor from which all floral organs have been evolved.

Is it possible to go still farther? In a comparable case—an intensive study of the malformations of maize following attack by corn smut (*Ustilago maydis*)—Iltis (22) has found structural evidence which he considers to indicate beyond doubt that *Zea* has been derived indirectly from the Andropogoneae. This might arouse expectations that these malformations of teosinte may offer some structural evidence of significance in the phylogenetic question of the ancestry of teosinte and even of the ancestry of maize, with which teosinte is more or less involved in the hypotheses advanced by Collins

(10, 12, 15), Montgomery (36), Weatherwax (53), and others. Yet toward a solution of the disputed question whether maize originated by slow evolution or as a mutation or sport from teosinte or some ancestral plant like it; or whether it arose as the result of hybridization between teosinte and some other grass; or whether pod corn, erroneously believed to grow wild in Paraguay, is the ancestor of cultivated maize quite separate from teosinte, these abnormal inflorescences of teosinte seem to furnish no clear evidence.

To be sure, these malformed teosinte spikelets somewhat resemble those of pod corn (*Zea tunicata*) in the overgrowth of their glumes, a character well developed in this type of *Zea*, not only in the ear but also at times in the tassel. (Collins 11, pl. 13, B). This resemblance, even though some consider pod corn as the type most primitive or most like ancestral maize, can hardly be of any special significance, however, for similar overgrowths of floral parts occur in such genera as *Pennisetum* and in other tribes too remote to furnish any evidence of ancestral characters or relationships in the Maydeae. Likewise, there seems to be no significance in the resemblance between the adherence and binding of the leafy parts in certain deformed teosinte spikelets (fig. 2, C, D) and that traced by Kempton (27, fig. 16) as an inherited feature in "adherent" plants of certain strains of maize, and between the lobing and consequent entanglement of parts in the deformed teosinte tassels and that which Kempton (26) has found in leaves, husks, and tassel glumes, not only in maize but also in teosinte and related grasses.

Moreover, Collins (14) has suggested that the perennial type of teosinte is the more primitive from which the annual has been derived (possibly by crossing with its annual relative, maize). The unusually abundant and prolonged production of new shoots by the infected teosinte plants might be interpreted as a reversion to an ancestral condition. More probably, however, it is merely an example of the prolongation of vegetative growth which often follows tolerated parasitism of *Sclerospora* in other hosts (57, pl. 5).⁴

In the fifth place, these malformations in teosinte present interesting comparisons to those recorded in cases of infection by the other species of *Sclerospora* in numerous grasses. In the case of the common *Sclerospora graminicola*, for example, malformations of the inflorescence of an extensive list of grasses, chiefly in the tribe Paniceae, have been reported many times from Europe, Africa, China, Japan, and India. Also, the widely distributed *S. macrospora* has been found inducing deformities of the floral structures in some 25 hosts (including maize, wheat, rice, and many wild grasses) in 9 of the 11 graminaceous tribes in many localities through Europe, Asia, Australia, and the United States. In the other species of *Sclerospora*, moreover, although less numerous, striking, and widespread, similar cases have been reported, so that in all more than 40 papers might be referred to as pertinent. From these facts it seems obvious that these plants of teosinte, in showing malformations of the inflorescence as the result of attack by downy mildews of the genus *Sclerospora*, were only behaving toward these parasites as many other genera of grasses have

⁴ Further resemblances of possible phylogenetic significance might be noted, but realizing that such material can be better interpreted by others more familiar with the morphology, development, genetics, history, relationship, and agronomy of maize, teosinte, and their relatives, the writers will gladly turn over the remaining material, including slides, photographs, and notes, to those who will undertake further study.

been found to behave in the history of our knowledge of this group. On going over these cases and comparing the deformities in the tassels of teosinte with those developed in other male inflorescences, such as the tassels of maize (17, 19, 23, 25), and those formed in the perfect spikes or inflorescences of other grasses, such as *Pennisetum* (5, 8, 29), *Setaria* (20, 21, 29, 34, 48, 49, 51, 58), *Triticum* (1, 2, 4, 39, 40, 41, 43, 47, 52, 55), *Oryza* (18, 59), *Agropyron*, *Phragmites*, etc. (42, 43), it is noticeable that there is a general similarity, but that these in teosinte are on the whole more extensive in their growth, more elaborate and complex, and more profoundly altered in their structure. In their behavior, that is, in the partially successful growth of some of the more pronouncedly modified spikelets as if they were young independent plants, they are in some ways unique among the cases of abnormalities resulting from *Sclerospora* attack. It seems probable, however, that some of the elaborately malformed spikelets in the virescent tassels of maize infected with *S. macrospora* described in Italy by D'Ippolito and Traverso (25) and others would have made at least abortive attempts at independent apogamous growth if they had been given opportunity.

Finally, when the foregoing points have been considered, what seems to be the most probable interpretation of such malformations as these in teosinte? All deformed inflorescences in the case of wild and cultivated Gramineae are not occasioned, of course, solely by the growth of *Sclerospora* in the host tissue. Deformities with general resemblance to those just considered have been found, for example, to develop as the result of attack by nematodes in wheat (Leukel, 30) and rice (Butler, 7); of infestation by insects in wheat and *Setaria* (D'Ippolito, 24); of chemical and mechanical injury in maize (Blaringhem, 3); and even, apparently, of excessive moisture in wheat (Lo Priore, 31); probably following the general principle emphasized by Knox (28) that most malformations such as phyllodies, multiplications, fasciations, torsions, or virescences arise from injuries and are not heritable. Also, in a few instances malformations of the inflorescences very similar to these of traumatic origin have been found by Kempton (27), Zapparoli (60), and others to develop in maize unassociated with any detectable injury and to persist as hereditary characters.

These points seem to suggest the following: Under normal conditions the floral parts of teosinte and other grasses have inherent possibilities of certain structural development regarded as usual, normal, or typical. Also, however, they have inherent potentialities for ways of development that are unusual, nontypical, or abnormal, but may be induced by the action of various factors that are injurious or that disturb the usual course of growth. Obviously, there are limits to the inherent capabilities for abnormal development; but within these limits they show great range in such structural excesses as remarkable leaflike elaboration, extensive overgrowth of floral envelopes, and suppression of sexual organs; and these abnormalities, agreeing in general, may be called forth in response to various diverse disturbing influences. Under ordinary conditions of growth in the field, however, such disturbing influences are encountered but seldom; consequently, such malformations develop only rarely. Under ordinary circumstances, also, cases of malformation attributable to

chemical injury, to harmful environmental conditions, or to noxious insects are relatively infrequent.

Attack by *Sclerospora*, however, is a disturbing factor encountered in such a wide range of valuable and much-studied host crops, in so many different parts of the world, and it is so commonly followed by malformation of the inflorescence, that such deformation very naturally has come to be regarded as one of the distinctive symptomatic stigmata of these diseases.

SUMMARY

This paper considers the case histories, structural peculiarities, and possible interest or significance of malformed male inflorescences of teosinte (*Euchlaena luxurians*) infected with the downy mildew (*Sclerospora philippinensis*) in the Philippines.

The tassels were strikingly altered in appearance and structure; the spikes shorter, the rachis in extreme cases comprising only a few joints; the floral elements progressively reduced in size and number from base to tip of spikes, the tip spikelet in some represented by a single glume; the glumes, and particularly the lemmas, in reduced spikelets, excessively hypertrophied, the latter usually characteristically enwrapping although occasionally expanded above to a green blade, leaflike in gross structure and in histology; the paleas less hypertrophied, sometimes in the second florets only vestigial; the stamens generally wanting, but when present having atrophied anthers and abortive pollen. Not only were the tassels profoundly malformed, but the female inflorescences were suppressed, so that the plants were completely sterile. This sterility was accompanied by unusually prolific vegetative growth, the continued and abundant production of new shoots showing some resemblance to the growth habit of perennial teosinte.

In tassels left lying on the ground during heavy rains, deformed spikelets comprising leafy shoots resembling seedling plantlets remained green and vigorous, grew into an upright position, sent out roots from their bases, and when transplanted continued to develop independently for more than a week. Within their tissues the mycelium of the fungus remained living and apparently capable of continued growth had the plantlets lived. As such cases of apogamy or vivipary are unusual in the gramineous tribe Maydeae and exceedingly rare in teosinte, these are described and illustrated, and compared with those following infection by other species of *Sclerospora* in other grasses.

The possible bearing of these malformations on the general question of the interpretation of floral malformations is outlined and their possible significance is considered.

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PROPAGATION AND FOOD TRANSLOCATION IN THE COMMON MILKWEED¹

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INTRODUCTION

The recent interest in the milkweed (*Asclepias syriaca* L.) has centered around its possible commercial use. It has been investigated as a possible source of rubber from its latex, oil from its seeds, fiber from its vascular elements, and coma from its fruit. Little attention has been given, however, to the movement of food materials within the plant, and where it has been studied the major interest has been confined to changes which occur within the individual organs, such as the root, leaf, stem, or fruit.

The present investigation was undertaken to gain an understanding of certain physiological processes which affect successful propagation, together with the formation of various organic constituents and their translocation at frequent intervals throughout the vegetative and dormant period.

REVIEW OF LITERATURE

In the milkweed, vegetative propagation is accomplished by adventitious buds appearing along the surface of a deeply penetrating root system (26).³ In the edible canna, Ripperton and Goff (31) contended that vegetative viability depends upon the condition of the adventitious bud rather than upon the root stalk proper.

According to Crocker (6), weed seeds have a high vitality, especially the more noxious weeds and those in which the seed coat secures a long delay. However, this is contrary to the statement of Duvel (10) and others, who failed to emphasize the seed-coat characters in their study of the vitality of weed-seed embryos. Imperviousness of seed-coat characters to oxygen and to moisture has been stressed in the work of Crocker (6, 7), Shull (36), and Harrington (15, 16, 17), while Pack (25) has shown that proteins decrease in the seed coats during afterripening. Merlis (21), Schmidt (33), and Kirkwood (20) have indicated a transformation of oil to sugar in fatty seeds. Certain seeds, such as the guayule (20) and hawthorne (6), however, fail to germinate under any circumstances until after a long period of dormancy. In such instances the embryo is regarded as immature, and certain changes prior to germination are required.

In spite of the wide natural distribution of the common milkweed, no record has been found relative to the germination of its seed. In the present paper attention is confined to the germinational response of milkweed seed under various environmental conditions.

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³ Reference is made by number (italic) to "Literature cited," p. 849.

Studies upon the elaboration and movement of food materials in plants have been approached from several different points of view. In the leaf tissue these phenomena have received the attention of Brown and Morris (4), who found sucrose the primary product of synthesis and hexoses the primary product of translocation. Sucrose predominated in young mangold leaves, while later hexoses increased throughout the entire leaf with an accumulation at maturity (8). The work of Parkin (29) on the snowdrop and of Colin (5) on the sugar beet substantiates the claim that sucrose is the first sugar formed. This sugar is then reconverted into hexoses for translocation to the root, and there in the presence of an enzyme it is converted to starch, inulin, or sucrose, as the case may be. Although starch is absent from the leaves of several plants (8, 9, 22), Schimper (32) concluded that hexose formation preceded that of starch and that the latter is only formed when glucose reaches a fixed maximum limit, which differs for each species.

Attention also has been given to the change in quantity and composition of food material translocated during different stages of maturity. Shaw and Wright (35) found total proteins decreased in both sunflower and corn throughout the growth period. While starch remained constant in the case of the sunflower, it increased twentyfold in the corn plant. The largest amount of carbohydrate and ash material was found to be present in the timothy plant during its early stages of development (13), while nitrogen and ether extract increased during maturity. Nitrogenous material was stored chiefly in the roots, but no starch was present during the storage process. In the wheat plant (13, 19, 39), nitrogen and carbohydrates appear to be the materials which the leaf and stem yield to the maturing head. During late fall as the sweetclover enters dormancy, nitrogen of the tops decreases and is stored in the roots (37). During active growth the largest amount of nitrogen was found in the tops, the maximum appearing during the month of June.

Although the milkweed possesses certain other commercial possibilities (24), interest in this plant has largely centered around its value as a source of rubber (11). Fox (12) indicated that the rubber content varies somewhat with the amount of available soil moisture, while Hall and Long (14) found that ecological factors affect rubber formation. No analyses, however, have been reported concerning changes in rubber content with progressive maturity of the plant.

In the present investigation it was deemed advisable to follow the movement of organic and inorganic materials in the milkweed by making separate analyses of the roots, stems, and leaves at stated intervals over a period of one year. Movement of food reserves in the different organs could thus be correlated with each other as well as with progressive maturity of the entire plant.

EXPERIMENTAL STUDIES

PROPAGATION BY SEED

Preliminary observations were conducted upon native plants, but it soon became apparent that it would be necessary to propagate the milkweed in order to obtain a sufficient supply of experimental material. For this work a plot, comprising approximately 100 square feet, situated 2 miles south of Ames, Iowa, was selected.

During the fall of 1925 a pint of seeds was collected from native plants. These were dried in paper bags in the laboratory until the following spring, at which time they were scarified. There occurred an average germination of 85 per cent. During the week of May 1, 1926, a pint of scarified seed was used to plant about one-twentieth of an acre in rows 3 feet apart. Because of excessive drought germination was slow, but by June 15 the young plants had attained a height of 5 inches and required thinning. During the first year five plants of the entire lot blossomed and produced seed, thus showing unmistakably that they were annuals. These were noted and the seed from them was collected separately the following year. A

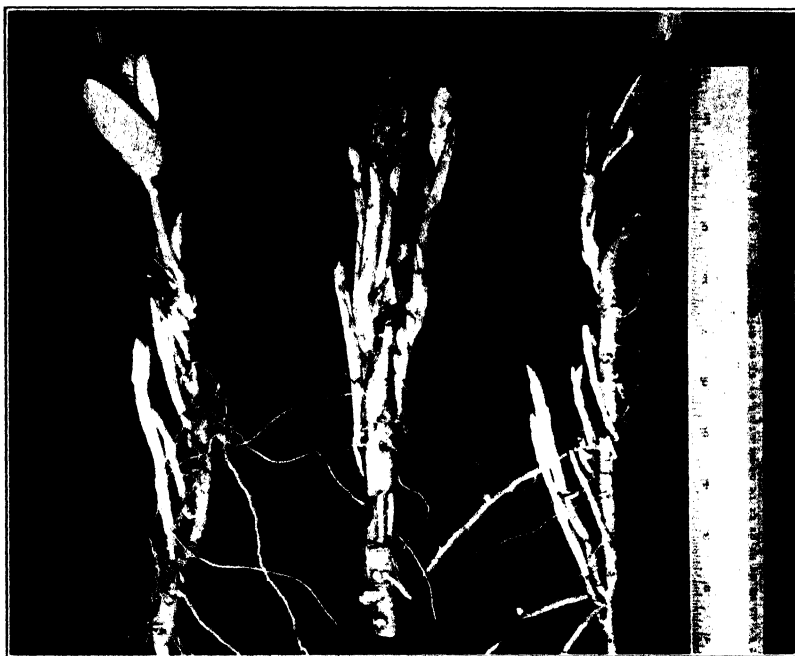


FIGURE 1.—Milkweed plants dug at Ames, Iowa, May 4, 1927. Vegetative propagation occurs from the previous year's roots

killing frost during the first week of October, 1926, resulted in the shedding of all leaves and the cessation of further growth and development.

In the spring of 1927 active growth of the plants in the field plot, as manifested by their appearance aboveground, began early in May. The condition and mode of formation of new plants may be seen in Figure 1. A large number of adventitious buds appear on the rootstocks of the previous year. In the breaking up of these older rootstocks, the distribution of the milkweed may actually be increased instead of curtailed. Figure 2 shows the field plot, on July 14 during the late-blossom stage. Sterility resulted from attempts to self-pollinate the five annual strains previously mentioned. Cross-fertilization by such agencies as wind and insects evidently is the rule.

SEED GERMINATION

Detailed studies were made on the germination of seed under different conditions. Prior to a killing frost, seed was collected from native milkweed grown during the summer of 1925. When placed in germination chambers the fresh seed showed little response. Rapid germination was not attained until after the seed had been stored for approximately four months. This fact indicates that milkweed seed must pass through a rather protracted period of afterripening.

To determine the practice necessary to obtain maximum germination, a study was made of the germination of the seed as influenced by chemical treatment, temperature, and storage. In some instances the seed used had been stored in the laboratory for 12 months, in others for only 2 months. Its response to temperature, seed-coat mutilation, and oxygen is summarized in Table 1.

TABLE 1.—*The effect of treatment and storage upon germination of milkweed seed*

Specific treatment	Temperature during time of germination	Period of storage	Germination
	° C.	Months	Per cent
None.....	20-25	12	21
Do.....	20-25	2	14
Kept at 10° C. for 5 days.....	20-25	12	32
Kept at 10° C. for 8 days.....	20-25	2	14
Exposed to concentrated H ₂ SO ₄ for 3 minutes.....	20-25	12	40
		2	23
Exposed to concentrated H ₂ SO ₄ for 6 minutes.....	20-25	12	54
		2	20
Scarified.....	20-25	12	75
		2	24
Scarified, and kept at 10° C. for 5 days.....	20-25	12	92
		2	40
Kept in an atmosphere of oxygen.....	20-25	12	46
		2	12
Kept in an atmosphere of oxygen 10° C. for 5 days.....	20-25	12	59
		2	42
Oxygen scarified.....	20-25	12	85
		2	24
Oxygen scarified, and kept at 10° C. for 5 days.....	20-25	12	91
		2	70

A study of this table shows that milkweed seed under natural conditions possesses low germinating power even though the vitality of the embryo may remain high. Seeds that are completely after-ripened give a decided response to scarification; but only a slightly higher germination was attained in an atmosphere of oxygen. It may be concluded, then, that mutilation of the testa increases the imbibitional capacity of the embryo for moisture rather than for oxygen. Alternating temperatures favor germination even in after-ripened seed. This is true also after scarification, and indicates that in milkweed the change caused by alternating temperatures is not confined to the seed coat alone but is shared also by the embryo.

No decided response to scarification was noted in seed stored for two months and germinated at room temperatures. Evidently the seed-coat characters alone are not responsible for the long period of afterripening. In this case 14 per cent of the untreated seed germinated at room temperature and no increase was obtained from

scarification. However, when the seed was subjected to increased oxygen supply and alternating temperatures together with scarification, the germinational response rose to 70 per cent. In general low temperatures inhibit respiration, producing a low respiratory ratio which permits a storage of oxygen in the seed and tends to hasten afterripening. Afterripening is also hastened by increased oxygen pressure, alternating temperatures, and scarification.

VEGETATIVE PROPAGATION

In the fall of 1925 a number of plants were lifted from the soil with as much of the root adhering as was possible and taken to the greenhouse, where a medium temperature was maintained. The first plants were transferred on October 3. Additional plants were dug



FIGURE 2.—Experimental milkweed plot, blossom stage, Ames, Iowa, July 14, 1927

on November 5, December 4, and on January 18 of the following year. The roots were placed in 7-gallon earthenware jars. One-half of the plants were set in a sand medium and the remainder in clay loam. In the October root collections a period of six weeks elapsed before growth began, while in the subsequent propagations the time interval became proportionately shorter, until in the January plantings only two weeks was required. These results suggest that the roots undergo a well-defined rest period. No attempt was made to shorten the rest period by the use of chemical reagents. In all cases the plants in sand cultures responded more rapidly to treatment than those in the clay-loam medium. In both series the amount of stored food material in the roots was the same. The greater growth response in the sand cultures was due possibly to better aeration. Normal growth, however, was not attained in the greenhouse, and when the plants were transplanted to an out-of-door plot in the spring they

were no further advanced after a few weeks than those which came up under normal environmental conditions. The greenhouse procedure, therefore, offered little encouragement as far as increased development was concerned. Since the roots of the milkweed undergo a definite period of rest, vegetative propagation is possible, without an appreciable loss of time, from roots which have been allowed to remain out of doors all winter.

FOOD TRANSLOCATION IN THE PLANT

A study was made of synthesis and translocation and storage of food materials to ascertain the changes in these processes that take place in the plant at different stages of maturity. Chemical analyses of plants were made at definite intervals. The plants used had been grown the previous year from native seed. Samples of the roots were collected once a month from November, 1926, to November, 1927, inclusive. Samples of the green portion of the plant were collected at stated intervals from May, 1927, to November, 1927, inclusive. The plants succumbed to a killing frost in the early part of October. The samples taken in November were therefore indicative of post-mortem composition.

CHEMICAL ANALYSES

Immediately after collection the samples were brought to the laboratory, cut into 1-inch pieces, placed in a Freas electric vacuum oven, and desiccated at 65° C. in a current of air. The air-dried material was ground until it could be passed through a 100-mesh sieve. Chemical analyses were made upon this material by the methods described below.

MOISTURE DETERMINATION.—A tared sample of ground tissue was dried to constant weight in a Freas electric vacuum oven at 70° C.

FAT.—The residue from the moisture determination was freed from lipoids and soluble pigments by percolation with anhydrous alcohol-free ether. The ether extract was dried to a constant weight in an electric oven at 100° C. and expressed as percentage fat.

REDUCING SUGARS.—After expulsion of the ether, the residue of the sample was extracted with boiling 90 per cent alcohol (to which a small amount of CaCO₃ was added) for one-half hour on a hot plate. The filtered extract was concentrated to remove alcohol, diluted with water, defecated with neutral lead acetate, freed from excess lead by sodium carbonate, and finally made up to definite volume. Reducing sugars were determined on aliquot portions by the Quisumbing and Thomas (30) method and the results expressed as percentage dextrose.

TOTAL REDUCING SUGAR.—Aliquot portions from the original sugar extract were hydrolyzed with boiling 2.5 per cent HCl for one hour. Total reducing sugar determinations were carried out and the results expressed as in the case of simple reducing sugars.

STARCH AND DEXTRIN.—The residue from the sugar extraction was boiled with 150 c. c. of water for two minutes in order to gelatinize the starch. After being cooled to 38° C. and digested with fresh saliva until a negative result was obtained with iodine, the filtered solution was hydrolyzed with 2.5 per cent HCl for 2.5 hours. Glucose was determined as above. The results were multiplied by the factor giving the equivalent values for starch and dextrin and expressed as percentage of starch. This figure actually includes the amount of water-soluble pentosans and any other soluble or partially hydrolyzed products possessing reducing power.

HEMICELLULOSE OR ACID-HYDROLYZABLE MATERIAL.—The residue from starch digestion was boiled with 150 c. c. of 2.5 per cent H₂SO₄ (by weight) for one hour. The filtered solution was neutralized and clarified in the usual manner. The

reducing power of this group of carbohydrates was determined (30) and expressed in percentage of glucose.

ASH.—A 2-gm. sample was ashed at a dull red heat in an electric furnace to a constant weight.

PENTOSE.—Two-gm. samples were distilled with 12 per cent HCl according to the Official Methods (1). The furfural phloroglucid value was multiplied by the proper equivalent and expressed as percentage pentose.

TOTAL NITROGEN AS PROTEIN.—The total nitrogen present in a 2-gm. sample was determined by the Kjeldahl method and the result multiplied by the factor 6.25 to represent total nitrogen expressed as crude protein.

ACETONE EXTRACT.—A 2-gm. sample was extracted 24 hours on an electric hot plate with chemically pure acetone. The weight of the evaporated extract, containing gums, resins, and pigmented material, was expressed as percentage acetone extract.

BENZENE EXTRACT.—The residue from the acetone removal was extracted 24 hours on an electric hot plate with chemically pure benzene. The soluble product (rubber) was expressed as percentage benzene extract.

In the presentation of the chemical data all percentages are calculated upon air-dry basis.

The data pertaining to the chemical composition of the leaves and stems are presented in Table 2 and in Figures 3 and 4. The total nitrogen content of both leaf and stem tissue of the young, succulent plant remains high, that of the leaf surpassing that of the stem during May and June. With an increase in maturity the leaf loses more than 70 per cent and the stem 82 per cent of its nitrogenous constituents. Although the ash content of the stem decreases during the summer months, inorganic constituents again accumulate during early fall. The leaf shows a progressive increase with maturity, the ash content being more than twice as great in November as in May. The amount of gum and resins, as indicated by the acetone extract, represents from 15 to 25 per cent of the total dry weight of the tissues. These substances are found in larger amounts in the green portion of the plant, predominately in the stem.

TABLE 2.—Percentage composition of milkweed leaves and stems during growth cycle

LEAVES

Date of sampling	Moisture	Total nitrogen as protein	Ash	Acetone extract	Benzene extract	Reducing sugar	Total sugars	Starch ^a and dextrin	Acid hydrolyzable material	Fat	Pentoses
May 15.....	2.58	32.55	8.90	14.45	0.49	4.83	15.10	0.95	5.48	5.75	9.52
June 5.....	2.20	29.39	9.50	13.75	.60	5.84	13.02	1.75	6.52	6.30	10.53
June 23.....	2.75	17.50	10.90	15.00	.81	5.28	11.28	.95	7.40	6.40	12.13
Aug. 5.....	3.50	13.45	14.10	13.45	1.50	2.17	8.48	.00	6.50	8.18	10.55
Sept. 7.....	2.30	12.77	16.60	14.68	2.55	1.95	8.50	1.08	5.48	10.45	10.02
Oct. 6.....	2.50	10.93	17.30	12.98	2.85	.68	3.70	1.07	7.76	11.35	12.03
Nov. 10.....	2.35	15.75	19.10	11.20	.82	1.80	3.74	2.49	8.28	8.00	10.73

STEMS

Date of sampling	Moisture	Total nitrogen as protein	Ash	Acetone extract	Benzene extract	Reducing sugar	Total sugars	Starch ^a and dextrin	Acid hydrolyzable material	Fat	Pentoses
May 15.....	2.26	23.50	8.45	22.70	0.30	15.68	20.56	1.27	7.49	4.40	10.02
June 5.....	3.72	15.05	7.00	25.60	.27	15.08	21.88	.92	7.60	3.25	13.20
June 23.....	3.18	9.72	5.15	20.25	.47	9.68	16.48	1.40	10.00	3.05	15.82
Aug. 5.....	3.45	6.56	2.80	18.30	.40	6.88	10.20	.32	11.48	2.40	19.06
Sept. 7.....	2.85	5.05	3.10	17.14	.30	8.25	14.08	.75	11.04	2.50	20.33
Oct. 6.....	3.00	3.97	4.50	12.20	.31	5.02	7.13	1.30	14.72	2.15	21.25
Nov. 10.....	3.65	4.20	5.70	7.50	.30	.88	1.00	.00	15.76	2.75	21.64

^a No iodine reaction for starch, the small amount of reducing substances here represent dextrans and H₂O soluble pentosans.

Rubber (benzene extract) is present in the stems only in traces. Since none is present in the roots, the leaf must remain the center of rubber synthesis and storage. This conclusion is further strengthened by the fact that there is a gradual increase in benzene extract with maturity. Attention should be called to the post-mortem examination as represented by the samples obtained November 10 in which

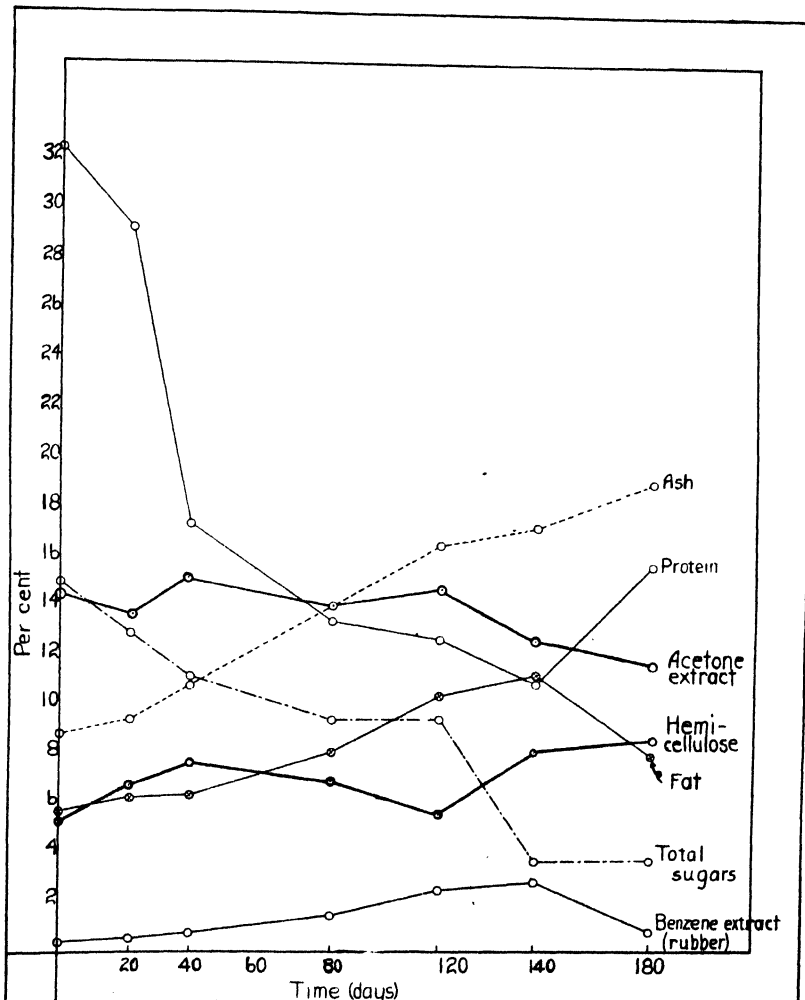


FIGURE 3.—Changes in chemical composition of milkweed leaves during the growth cycle

over four-fifths of the total rubber present had been destroyed by a killing frost. The analyses showed 2.53 per cent rubber in leaves prior to killing frost, 1.54 per cent present five days after the frost, and 0.50 per cent present two weeks after the frost. It is difficult to explain this loss, since no increase was found in the acetone or benzene extract of the stem tissue.

Like nitrogen, soluble carbohydrates appear to reach their maximum concentration in the young tissue. A progressive loss of these soluble constituents was noted. According to Parkin (27), starch as a rule is formed plentifully in most dicotyledonous leaves. In the milkweed, however, this was not found to be the case. Evidently the milkweed stores its leaf carbohydrates in the form of sucrose, since this sugar predominates in the leaves. In the stem the predominating sugar is hexose. The total sugar content of the stem is also considerably higher than that of the leaf. Hemicelluloses evidently do not serve as a reserve or storage material in the milkweed leaf, but in the stem they increase with the age of the plant. Approximately 10 to 12 per cent of pentosan material is found in the leaf. The absence of starch and the fairly uniform hemicellulose and pentosan content in the leaf are strong indications that synthesis,

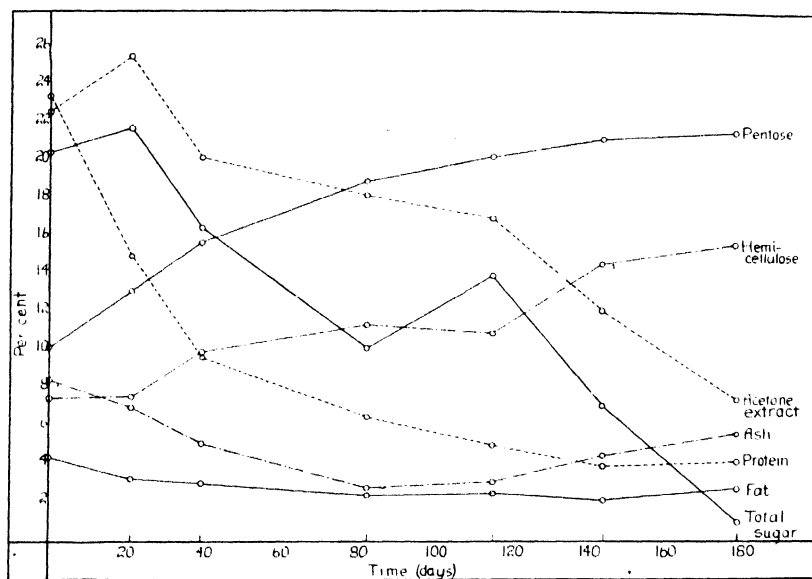


FIGURE 4.—Changes in chemical composition of milkweed stems during the growth cycle

storage, and translocation of carbohydrate material are accomplished through the medium of sucrose.

The results of chemical analyses of the root tissue made at monthly intervals during the year are shown in Table 3 and Figure 5. It is evident that the root system serves as a storage medium for vegetative propagation. Here, as in the leaf and stem, nitrogenous and carbohydrate materials form the chief migratory and storage constituents. The gums, resins, and lipoids are not concentrated in the underground organs and exert probably only a minor influence. Nitrogen migrates out of the root tissue during the period of great physiological activity in the green portions of the plant, and accumulates during the late fall and winter months. Although Murneek (23) found hemicellulose to function as a storage material (like starch) in the spur tissue of the apple tree, nevertheless it does not offer such a possibility here since

no decided changes occur in its deposition or utilization. The pentosan content of the root constitutes from 12.5 to 19.65 per cent of the dry weight. Except during the summer months, it shows but little fluctuation. This type of material appears as a storage constituent in the stem only.

TABLE 3.—Percentage composition of milkweed roots during growth cycle ^a

Date of sampling	Moisture	Ash	Total nitrogen as protein	Acetone extract	Fat	Reducing sugars	Total sugar	Starch and dextrin	Acid hydrolyzable material	Pentos
Nov. 3	4.10	7.35	9.18	5.90	2.20	0.52	14.08	16.38	9.26	13.84
Dec. 1	3.60	6.30	10.50	6.15	2.10	1.50	18.72	8.56	9.00	15.85
Jan. 8	2.80	8.50	11.17	6.40	2.95	1.40	19.06	6.19	8.10	15.65
Feb. 10	4.00	6.78	11.59	6.70	2.68	1.30	21.84	8.71	8.91	14.43
Mar. 12	4.00	6.60	11.17	6.40	2.40	1.45	21.12	8.97	10.12	16.00
Apr. 10	4.00	7.50	12.77	7.00	2.33	2.30	18.72	8.38	9.52	15.95
May 4	3.40	7.00	12.34	6.35	3.22	1.41	13.40	10.00	8.64	16.00
June 5	3.00	8.18	9.88	8.35	3.35	3.20	8.60	6.80	9.00	19.65
July 1	3.30	9.80	5.60	8.60	4.00	3.00	8.50	11.70	9.52	18.70
Aug. 5	2.60	9.85	4.81	8.30	3.75	3.13	10.04	13.40	8.84	18.16
Sept. 7	3.00	7.00	4.72	5.90	2.50	2.12	9.67	30.56	8.70	14.34
Oct. 7	4.30	7.12	7.80	5.55	2.50	.88	10.56	23.44	8.00	12.50
Nov. 7	2.40	8.70	9.21	6.30	2.80	.80	17.28	15.20	8.60	15.15

^a The benzene extract was nil in all samples.

It is in the root that starch is first encountered. This fact appears significant because this reserve carbohydrate is found in neither the leaf nor stem tissue, where rubber is present. Sucrose here, as in the leaf, remains the predominating sugar. Starch begins to accumulate during early midsummer and reaches its maximum in September. During the winter months it is converted to sucrose and attains its highest concentration during February and March. This fact is substantiated by the statement of Hooker (18) and Blackman (3) that the inception of dormancy is usually preceded by a carbohydrate and nitrogen increase in which sucrose and starch present a definite interrelationship. During active growth in the green portions of the plant, ash and rubber are stored in the leaf, hemicellulose and pentosans in the stem, and starch in the root.

Hexose sugar is present in the root only at a low concentration, and the amount fluctuates but little with change of season. Sucrose, on the other hand, varies inversely with starch deposition. The starch which accumulates during the summer is converted to sucrose and is used for the production of new tissue the following spring. Figure 5 indicates the cyclic seasonal interrelationship between total sugars, starch, and nitrogen. Figures 3 to 5 show that during May and June the available carbohydrates are largely trans-located out of the root into the green portions of the plant. Nitrogen is also present in smaller amounts. During June and July flower-bud formation and fertilization occur, and in the period of great physiological activity that accompanies these processes carbohydrate and nitrogenous materials migrate out of the root, leaf, and stem tissues into the floral organs, there to be stored later in the developing seed.

COMPOSITION OF LATEX

The lactiferous system of the milkweed plant is composed of individual latex cells, and structurally is identical with that of the

Urticaceae and Apocynaceae. While little is known regarding the function of such a system, Parkin (28) has indicated that it serves in the translocation and storage of food reserves. Weiss (38) and Schullerus (34), on the other hand, attach little physiological sig-

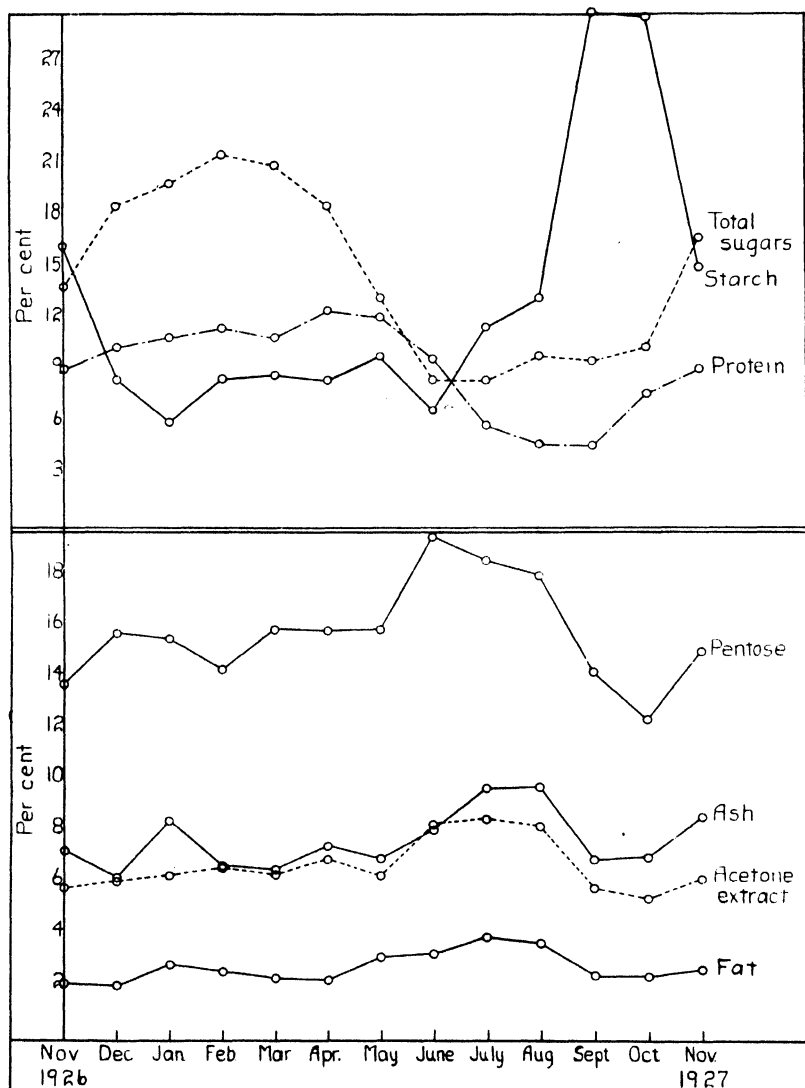


FIGURE 5.—Changes in chemical composition of milkweed roots during the growth cycle

nificance to its presence, believing that it serves merely as a receptacle for the waste products of metabolism.

It was not possible to follow the chemical changes that took place in the composition of milkweed latex during the growing season, since there were not enough plants available to furnish a sufficient

quantity for analysis. However, one collection of latex was made. To obtain the latex for chemical analysis a leaf or portion of the stem was ruptured at several places, and when a drop or two of latex appeared on the surface it was sucked into a small test-tube suction trap, and the rupturing and sucking process was repeated. About 30 plants and some four hours of labor were required for the collection of 20 c. c. of latex. Where the changes in rubber and resin content have been determined, analyses were made of the leaf and stem tissue as a whole. The chemical composition of milkweed latex, collected July 20, as compared with that of a 4-year-old Para rubbertree (*Hevea brasiliensis* L.), is shown in Table 4.

TABLE 4.—A comparison of the percentage chemical composition of latex from milkweed and from Para rubbertree

Milkweed	Constituent	Para rubbertree *
70.00	Moisture.....	70.00
1.40	Ash.....	.26
4.00	Total sugars.....	.79
23.00	Resins.....	1.22
3.34	Rubber.....	27.07
.46	Total nitrogen.....	.24
0	Disaccharides.....	
0	Dextrin and starch.....	
6.52	Hemicellulose.....	

* Beadle, C., and Stevens, H. (2).

It is easily seen from Table 4 that milkweed latex offers an inferior source of rubber. The moisture and total nitrogen content of the latex from the milkweed and Para rubbertree approach a common level. It is in the distribution of rubber and resins that a marked compositional difference lies. Milkweed latex contains about twenty times the amount of resin and only one-eighth the amount of rubber found in the exudate of *Hevea*. The ash and sugar values are from five to six times greater in the milkweed latex than in the Para rubbertree. From the above analyses it is evident that the secretory and compositional mechanism in the two species must be entirely different. Although sucrose is the predominating sugar present in the leaf, hexoses only are found in the latex. Since the latex for analysis was obtained from the midrib and petiole of the leaf and also from the stem proper, in which hexoses predominate, it appears that the laticiferous system might serve as a translocation medium for simple carbohydrates. Nitrogen was present only in small amounts.

SUMMARY

The common milkweed may be propagated vegetatively, but because of a well-defined rest period in the roots this method does not lend itself to practical use. The seed also requires a prolonged period of afterripening. This process is induced by embryological immaturity rather than seed-coat hindrance. Seed-coat permeability is a limiting factor in the germination of fully afterripened seeds. Moisture rather than oxygen is excluded. Alternating temperatures between 10° and 25° C. produced increased germination.

In young, succulent growth nitrogen and carbohydrates predominate. Sucrose is stored in the leaf, translocated in the form of hexoses through the stem to the root, and stored as starch during the late summer, only to be converted back to sucrose at the inception of winter. Leaf, stem, and roots yield nitrogenous and carbohydrate material to the developing flower and seed. Ash and rubber are stored in the leaf, pentosans and hemicelluloses in the stem, and starch and nitrogen in the root. No starch is present in the green portions of the plant.

From the limited trials made, it appears that gums and resins are present in the latex of milkweed in large amounts, rubber only to the extent of 3.5 per cent, and this is localized largely in the leaf. Freezing destroys more than four-fifths of the normal rubber present. A comparison of the latex of milkweed and *Hevea* indicates a decided compositional difference in the resin and rubber content. Hexoses appear as the only simple sugar present in milkweed latex, which suggests that these lactiferous structures may aid in carbohydrate removal from the leaf to the root, since hexoses are the types of sugar represented in the translocation form.

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EFFECTS OF SOIL TEMPERATURE AND REACTION ON GROWTH OF TOBACCO INFECTED AND UNINFECTED WITH BLACK ROOT ROT¹

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INTRODUCTION

Black root rot of tobacco, caused by the fungus *Thielavia basicola* (Berk. and Br.) Zopf, is known to be much affected by the reaction of the soil. In the field experiments of Anderson, Osmun, and Doran (3)² this disease did little or no damage in a soil as acid as pH 5.6, and it was severe in a soil with a pH value of 5.9. One year (1925), Anderson and Morgan (2) found that this disease was serious only in soils with a pH value of 5.95 or higher, but in the colder summer of the following year, they (14) found it in more acid soils and concluded that the safety point, at least for shade tobacco, may be nearer 5.6 than 5.95 on the pH scale. Anderson (1) concluded later that no injury occurs below pH 5.6, but that between pH 5.6 and 6.0 there is a doubtful zone within which the root rot may or may not occur.

Black root rot is known to be much affected by the temperature of the soil. Johnson and Hartman (10) found that it was most severe at 17° to 23° C., much less severe at and above 26° C., and practically negligible at about 30°. Valleau, Kenney, and Kinney (21) reported that at soil temperatures of 21° to 23° C. in the field, black root rot injured tobacco, but that at an average soil temperature of 25.5° C. tobacco grew fairly well in this soil.

In most if not in all previous experiments on black root rot of tobacco, the effects of soil temperature and of soil reaction on the disease have been considered as factors operating independently.

One of the objects of the work described in the present paper was to determine to what extent, if at all, the pH value of the soil at which black root rot of tobacco becomes measurably injurious is affected or changed by the temperature of the soil; in other words, to study the joint effect of these two factors of the environment.

There was in the beginning some reason to expect that the effect of a certain soil reaction on black root rot might itself be influenced by the temperature of the soil; for, according to Jones, Johnson, and Dickson (12), the optimum for an environmental factor in infection of a plant by a fungus may vary through some range with other simultaneously varying factors, and Faris (7) found that, in the case of infection of barley by covered smut, soil reaction and soil temperature modified the effects of each other. Furthermore, it was suggested by Morgan and Anderson (14) and by Anderson (1), on the basis of observations in the field, that the exact point on the pH scale at which black root rot of tobacco is a cause of loss may be influenced by the season and especially by the temperature.

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² Reference is made by number (italic) to "Literature cited," p. 871.

A clear understanding of the effect of environmental factors on black root rot of tobacco, or on any other plant disease, is possible only if the effect of these environmental factors on the host itself is known. The effects of soil temperature and of soil reaction on the growth of tobacco in the absence of *Thielavia basicola* were therefore investigated also, and the results obtained are here reported.

METHODS

In pot experiments, subsequently described, the constant soil temperatures desired were maintained by means of soil temperature tanks of the type described by Jones (11) but modified at the Massachusetts Agricultural Experiment Station. At each temperature there were several pots of soil, each of a different pH value.

For the experiments with *Thielavia basicola*, soil (a stony, sandy loam of glacial origin) was obtained from a field known to be infested with this fungus. Some of the plots in this field had been limed three years before and others had not. Soils of several different pH values were thus available. To secure an even greater range of pH values, the reaction of the soil for some pots was further adjusted with hydrated lime or with sulphuric acid. The amounts of lime or acid necessary to add to produce the desired changes in pH value were determined in preliminary experiments by methods essentially the same as those described by Hopkins (9) and Faris (7). The pH values were checked monthly,³ and were found not to change significantly during the course of each experiment.

In three series of experiments soil infested with *Thielavia basicola* was used. In two series of experiments soil sterilized by steam to rid it of this fungus was used in order to study the effect of soil environment on the growth of tobacco in the absence of black root rot.

In all pot experiments, a strain of Havana tobacco susceptible to black root rot was employed. In some cases seeds were sown directly into the pots. If transplants were used, they were taken from sterilized soil to insure freedom from black root rot when set. In one field experiment, strains of root-rot-resistant and root-rot-susceptible Havana tobacco were compared to determine their response to lime in the presence of *Thielavia basicola*.

The results in all experiments are based on the degree of infection of the roots and on the growth, dry weight or leaf areas, of plants.

EFFECT OF TEMPERATURE AND REACTION OF SOIL INFESTED WITH THIELAVIA BASICOLA ON INFECTION OF ROOTS AND GROWTH OF PLANTS

In three series of experiments, unsterilized soil infested with the root-rot fungus was used. Before the results of these experiments are described, however, mention should be made of the effect of soil temperature and reaction on the germination of tobacco seeds and on the period of incubation of black root rot, since the responses in these two respects were essentially the same in each of the several experiments.

SEED GERMINATION

Soil reaction had much less effect on the germination of seeds than on the subsequent growth of the seedlings. Germination was not

³ Except where otherwise indicated, pH values were determined colorimetrically, by the method used and described by Anderson and Morgan (2).

significantly affected by soil reaction within the range of pH values used, pH 4.6 to 6.9, inclusive. This range extends, on the more acid side, below that reported by Van der Poel (15, 16), in whose experiments tobacco seeds germinated well at hydrogen-ion concentrations between pH 5.6 and 7.8.

The relation of soil reaction to germination was not affected by soil temperatures within the ranges used, that is, 15° to 30° C.

Soil temperatures had more effect on the length of time required for germination than on the percentage of tobacco seeds which germinated. The seeds germinated in 4 days at 30° to 21° C., in 6 days at 18° C., and in 10 days at 15° C. Germination, although delayed, was fair at the lowest temperature used, 15° C., which is near the minimum temperature for the germination of tobacco seeds, 13° C., as determined by Johnson and Murwin and reported by Russell et al. (17, p. 16-17). The best germination was at 30° C., the highest temperature used.

PERIOD OF INCUBATION

Black root rot lesions with *Thielavia basicola* sporulating on them were found in some cases when seedlings were only 2 to 3 weeks old, but no black root rot was found on seedlings less than 2 weeks old.

The colder the soil, down to 18° C., and the more nearly neutral the soil reaction up to pH 6.9, the shorter was the incubation period for black root rot, assuming it to begin in infested soil with the germination of the seeds.

In the more acid soils with pH values of 5.6 or lower, the incubation period was about 10 days longer than in the more nearly neutral soils with pH values of 5.9 or higher. At a given soil reaction, the incubation period was 3 or 4 days longer at soil temperatures of 24° C. or higher than at lower temperatures.

FIRST SERIES

In the first series of experiments black root rot was present only as a trace, or very mild in soils having a pH value of 5.0 or lower at all soil temperatures used, 15° to 30° C. In soils with pH values of 6.5 to 6.9 the disease was severe at soil temperatures of 15° to 24° C., mild or very mild at 27° C., and only a trace was present at 30° C. The degrees of infection of roots were as indicated in Table 1, in which are also recorded the relative sizes of plants, as determined by leaf areas, measured with a planimeter.

At soil temperatures of 15° to 27° C., inclusive, but not at 30° C., plants were much larger in the more acid soils, with pH values of 5.0 or lower, than were plants in the less acid soils, with pH values of 6.5 or above. The greatest differences in sizes of plants, between those in the more acid and those in the less acid soils, were at temperatures of 24° C. and lower.

Black root rot did not retard the growth of plants in soil with a pH value of 5.0 or lower at any temperature. Retardation of growth due to black root rot occurred in soils with a pH value of 6.5 or higher, and this retardation of growth was greatest at soil temperatures of 24° C. or below, less marked at 27° C., and not significant at 30° C. Even in soil as nearly neutral as pH 6.9, black root-rot infection was much lighter and resulting interference with growth much less at 27° C. and at 30° C. than at lower temperatures.

TABLE 1.—*Effect of soil reaction and temperature on black root rot and on growth of tobacco*

[First series]

Soil, pH value	Extent of root rot on tobacco plants of different sizes when grown in infested soil at a temperature of—											
	15° C.		18° C.		21° C.		24° C.		27° C.		30° C.	
	Relative leaf area ¹	Black root rot	Relative leaf area ¹	Black root rot	Relative leaf area ¹	Black root rot	Relative leaf area ¹	Black root rot	Relative leaf area ¹	Black root rot	Relative leaf area ¹	Black root rot
4.6	89	Trace	53	Trace	116	Trace	106	None	108	None	82	None
4.8	68	do.	58	do.	104	do.	58	do.	64	do.	62	Do.
5.0	100	do.	100	do.	100	do.	100	Trace	100	do.	100	Do.
5.0	247	Severe	146	Very mild	224	Very mild	396	Severe	517	do.	466	Do.
5.5	18	Severe	12	Severe	9	Severe	10	Severe	36	Very mild	69	Trace
6.7	11	do.	11	do.	7	do.	12	do.	34	Mild	73	Do.
6.9	9	do.	6	do.	1	do.	6	do.	33	do.	68	Do.

¹ Expressed as relative numbers with leaf areas at pH 5.0 as base.² Leaf area expressed in square centimeters.TABLE 2.—*Effect of soil reaction and temperature on black root rot and on growth of tobacco*

[Second series]

Soil, pH value	Extent of root rot on tobacco plants of different sizes when grown in infested soils at a temperature of —											
	15° C.		18° C.		21° C.		24° C.		27° C.		30° C.	
	Relative dry weight of plants †	Black root rot	Relative dry weight of plants †	Black root rot	Relative dry weight of plants †	Black root rot	Relative dry weight of plants †	Black root rot	Relative dry weight of plants †	Black root rot	Relative dry weight of plants †	Black root rot
5.0	120	None	73	None	80	None	103	None	106	None	97	None
5.2	123	Trace	132	Trace	125	do	134	do	100	do	103	Do.
5.6	100	do.	100	do.	100	do.	100	Trace	100	do.	100	Do.
5.9	{ 10.12	Severe	{ 10.86	Severe	{ 2.92	Severe	{ 5.10	Severe	{ 16.62	Moderate	{ 9.28	Very mild.
6.1	88	do.	22	do.	21	do.	22	Severe	33	do.	52	Mild.
6.3	67	do.	31	do.	18	do.	21	do.	34	do.	56	Do.
6.3	49	do.	26	do.	26	do.	29	do.	32	do.	47	Do.
6.5	70	do.	27	do.	17	do.	30	do.	37	do.	45	Do.

¹ Expressed as relative numbers with dry weights at pH 5.6 as base.² Weight expressed in grams.

In this series, the critical point for black root rot on the pH scale was between pH 5.0 and 6.5 at all soil temperatures at which tobacco will grow below 27° C.

SECOND SERIES

In the second series of experiments, soils with pH values of 5.0, 5.2, 5.6, 5.9, 6.1, 6.3, and 6.5 were used. As before, the soil temperatures were 15°, 18°, 21°, 24°, 27°, and 30° C. The degrees of infection of the roots and the dry weights of plants, the latter expressed in relative numbers, are recorded in Table 2.

Root infection was absent, or present only as a trace, in soils with pH values of 5.6 or lower at all soil temperatures. In soils with pH values of 5.9 to 6.5, infection was severe at soil temperatures of 15° to 24° C. inclusive, moderate at 27° C., and mild or very mild at 30° C.

At all soil temperatures growth was relatively good in soils with a pH value of 5.6 or lower and relatively poor in soils with a pH value of 5.9 or higher. Retardation of growth due to black root rot in these less acid soils was, as compared with growth in the more acid soils, greatest at soil temperatures of 18° to 24° C., less at 27° C., and least at 30° C. A soil temperature of 15° C. is too low for tobacco, and growth was poor at that temperature regardless of black root rot.

In this series, the critical region on the pH scale for infection by *Thielavia basicola* was between 5.6 and 5.9, and this region was not changed by soil temperatures lower than 30° C.

It has often been noted that tobacco plants infected by *Thielavia basicola* in the field tend to grow better or to recover partially as they become older, and this has been attributed to a rise in soil temperature with the advance of the season. While higher soil temperatures are of course important, observations made in this series indicate that some of this recovery may occur without a rise in the soil temperature as the plants become older.

Black root rot in the less acid soils, pH 5.9 to 6.5, retarded the growth of plants more when they were young than it did later. Leaf areas were measured with a planimeter when plants in this series were 5 weeks old and again four weeks later. The areas of leaves of plants in the less acid soils, in which black root rot was more severe, were, as compared with the areas of leaves of plants in the more acid soils (pH 5.6 or lower), as follows:

- At 15° C., first measurement 33 per cent; second measurement 33 per cent.
- At 18° C., first measurement 21 per cent; second measurement 34 per cent.
- At 21° C., first measurement 15 per cent; second measurement 43 per cent.
- At 24° C., first measurement 9 per cent; second measurement 49 per cent.
- At 27° C., first measurement 7 per cent; second measurement 55 per cent.
- At 30° C., first measurement 13 per cent; second measurement 67 per cent.

Although the soil temperatures were constant, black root rot was less injurious in its retarding action on the growth of older plants than on the growth of younger plants. High soil temperatures were not so effective in protecting seedlings against retardation of growth due to black root rot as were these same high temperatures when the plants became older.

THIRD SERIES

In the third series of experiments, soil reactions were so adjusted as to give the following pH values: 5.5, 5.6, 5.7, 5.8, 5.9, and 6.0. The soil temperatures used were the same except that 32° C. was substituted for the 30° C. of the previous series.

The degree of root infection and the average dry weights of plants in each treatment, the latter expressed as relative numbers, are recorded in Table 3.

No more than a trace of infection by *Thielavia basicola* was present in soil with a pH value of 5.5 or 5.6 at any soil temperature. There was infection in soils with pH values of 5.7, 5.8, 5.9, and 6.0, and the critical point for black root rot on the pH scale was affected somewhat by the soil temperatures. At 15° C. growth was reduced about one-third and infection was of moderate severity at pH 5.7. At 18° C. black root rot was mild and growth was reduced about one-fourth at pH 5.7, and at pH 5.8 the disease was severe and growth was depressed by half. At 21° and 24° C., black root rot did not cause injury at pH 5.7, but at pH 5.8 infection was moderately severe and growth was somewhat retarded, and at pH 5.9 infection was severe and growth was retarded by about one-half. At 27° C. infection was only moderate in any soil, but there was a growth depression beginning at pH 5.8 or 5.9. At 32° C. there was no infection and no interference with growth even in soil with a pH value of 6.0.

In this series, as in the previous one, the critical region for black root rot on the pH scale was between pH 5.6 and 5.9. In the coldest soil, 15° C., the critical point was pH 5.7. At 18° it was pH 5.7 or 5.8; at 21° and 24° C. it was pH 5.8; and at 27° it was pH 5.8 or 5.9. Above 27° C. black root rot did no damage.

The indications are that tobacco in this soil would be safe from black root rot at any temperature at which this crop will grow in soil with a pH value of 5.6. Only in a very cold season would black root rot cause loss in this soil when the pH value was 5.7. If this soil had a pH value of 5.8 or 5.9, black root rot would be expected to cause growth depression below soil temperatures of 27° C. At 32° C., this soil would be safe with a pH value at least as high as 6.0.

But one would probably not be justified in attempting to specify definitely for all soils the pH value at which black root rot might occur, for the disease is known to be affected somewhat by the temperature of the soil, and it is not unlikely that it is also affected somewhat by the nature of the soil itself.

RELATION OF BLACK ROOT ROT TO THE TEMPERATURE AND REACTION OF SOIL IN THE FIELD IN 1927 AND 1928

The observations previously recorded were, of course, based on constant soil temperatures, but in the field during the growing season of tobacco the soil temperature is far from constant.

To supplement information secured in constant-temperature tanks, there is always, as has been pointed out by others (12), need for observations on the correlation of soil temperatures and plant diseases in the field. While a record of air temperatures is of some value, at least in comparing one growing season with another, a record of actual soil temperatures in the field is highly desirable, for, as shown by Keen and Russell (13) and as was suggested earlier by the work of Stockbridge (19), organisms in the soil are living in a rather warmer environment than would be inferred from the air temperatures.

A record of soil temperatures, 5 inches deep, was obtained in July and August, 1927 and 1928, in a tobacco field at Amherst, Mass. The daily maximum and minimum and the hourly mean temperatures of this soil are given graphically in Figures 1 and 2.

For the present, it is probably safer to consider the hourly mean temperatures rather than the daily maximum or minimum tempera-

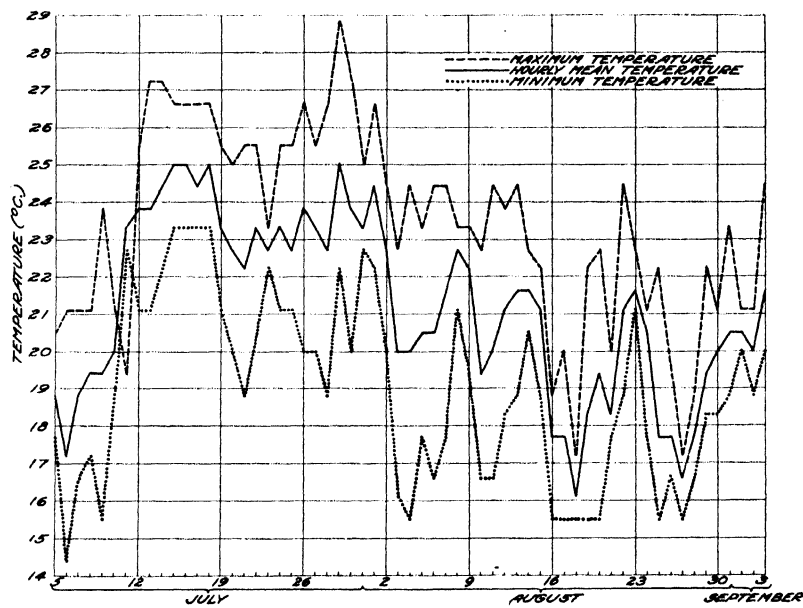


FIGURE 1.—Maximum, hourly mean, and minimum soil temperatures in field, summer of 1927

tures, although as has been pointed out by other investigators (12), the relative influence on plant diseases of daily maximum or minimum

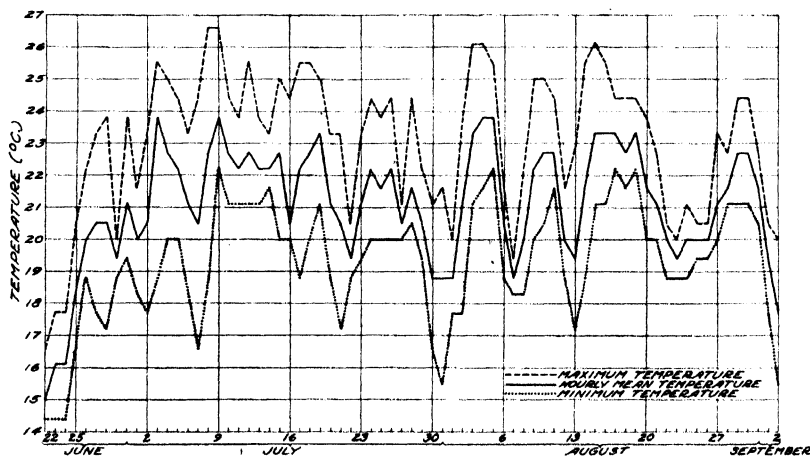


FIGURE 2.—Maximum, hourly mean, and minimum soil temperatures in field, summer of 1928

temperatures as compared with hourly mean temperatures is not known.

The pH values of the soil in this field during the growing seasons of 1927 and 1928 were 5.9 to 6.1. On the basis of results secured in

the temperature tanks and previously described, marked injury from black root rot would be expected in soil with these pH values if the soil temperatures were 24° C. or lower.

In 1927 the hourly mean temperatures of the soil in this field were below 24° C., and therefore low enough to favor infection by *Thielavia* from July 5 to September 3, with the exception of six days in July and one in August.

Under these conditions of soil temperature and reaction, root infection was severe. The yield per acre of cured leaf was only 987 pounds, while in adjacent plots the yield per acre was 1,659 pounds. In these plots the soil temperature was presumably the same as in the field, but the pH value of the soil was 5.6, too acid for black root rot.

In 1928 the hourly mean temperatures of the soil were below 24° C. from June 22 to September 2. As was to be expected, considerable black root rot developed, although it was not so severe as in 1927 in the same plots, probably because one more year had elapsed since this soil was limed (in 1923).

In 1927 the soil temperatures in the field were probably about normal in July and lower than normal in August, for the mean hourly air temperature at Amherst was 0.22° C. below normal in July and 1.98° C. below normal in August of that year. In 1928, however, the mean hourly air temperatures were not below normal at Amherst in either July or August. July and August in 1928, but not in 1927, were characterized by a total precipitation markedly greater than normal. So in these two years—one of slightly subnormal air temperature and approximately normal rainfall, the other of normal air temperature but excessive rainfall—the mean hourly temperatures of the soil were low enough (below 24° C.) to favor black root rot in this soil with a pH value of about 6.0.

EFFECT OF SOIL TEMPERATURE AND REACTION ON GROWTH OF PLANTS IN SOIL NOT INFESTED WITH *THIELAVIA BASICOLA*

In the experiments previously described, the higher the soil temperatures up to 27° to 30° C., the larger were the tobacco plants, and this was true in the practical absence of infection, that is in the more acid soils, as well as in the less acid soils on which there was black root rot. The optimum temperatures for the growth of tobacco (between 26° and 30° C. (12)) were, in the present experiments, about the same whether or not black root rot was present. Because of this, the condition of the roots, as well as growth measurements, was considered in determining the effect of soil temperature on black root rot.

The effect of soil reaction on the growth of tobacco in the absence of *Thielavia basicola* deserves consideration if we are to distinguish between soil reaction and the fungus itself as a cause of the poor growth of tobacco in soils with relatively high pH values. It is conceivable that the roots of tobacco plants might be more susceptible to infection if, in soil with a reaction approaching neutrality, the plants were weakened or their growth retarded by the too high pH value of the soil. But, according to the literature, the application of lime and the resulting higher pH value of the soil would benefit tobacco, at least in most soils of the Connecticut Valley, were it not for the presence of the black root-rot fungus. In the experiments of

Shchepkina (20) the optimum pH value for the growth of tobacco in solutions was 8.0. Briggs (4), working with a Connecticut Valley soil not infested with the fungus, found that tobacco made a better growth on limed than on unlimed soil. Chapman (6) concluded from his observations and experiments that liming is needed for the best growth of tobacco in Massachusetts. The immediate effect of liming an acid soil at this station was found by Anderson, Osmun, and Doran (3) to be an increase in the yield of tobacco, and not until a later year when black root rot developed were yields lowered. Morgan and Anderson (14) applied lime to an acid soil in Connecticut, and a marked increase in the yield of tobacco resulted.

It is of course possible to overdo the liming, and the unfavorable results on tobacco in such cases have been attributed, and with good cause, to the black root rot which follows. But evidently too much lime may be applied even when black root rot is not a factor. Johnson and Hartman (10) found that even in soil not infested with *Thielavia basicola* some lime increased the growth of tobacco, but that if more lime were added growth was retarded, at least at first.

In experiments by Slagg and his associates (18), the application of lime for three successive years was followed by lowered yields of tobacco, and meanwhile there was an increase in black root rot on limed plots; so the lowered yields might be attributed directly to the disease and only indirectly to the lime.

In order to secure further information on the effect of lime on tobacco, leaving out for the time the question of black root rot, the experiments described below were conducted.

EFFECT OF LIME ON STRAINS OF TOBACCO SUSCEPTIBLE TO BLACK ROOT ROT COMPARED WITH ITS EFFECT ON RESISTANT STRAINS

If a too high pH value of soil rather than increased infection by *Thielavia basicola* were the direct cause of trouble with tobacco in certain soils, as great a depression in yield would be expected in the case of strains of tobacco resistant to black root rot as in the case of susceptible strains in limed soils. That such is not the case is shown by the following results.

Havana tobacco of a strain susceptible to black root rot and Havana tobacco of two resistant strains⁴ were set in adjoining plots in a field known to be infested with *Thielavia basicola*. In the case of each strain, half the plots used had been limed heavily (a total of 5 tons per acre, the last of it applied three years previously), and these plots had a pH value of 6.0. The other half of the plots used had not been limed, and these had a pH value of 5.4.

Roots were examined at the end of the season. There was only a trace of infection in the more acid soil on any strain, susceptible or resistant. In the less acid soil, black root rot was severe on the susceptible strain and very mild or present only as a trace on the resistant strains.

The yields per acre of cured leaf and the percentage loss in yield of limed plots, as compared with the yield of unlimed plots, are recorded in Table 4, together with pH values of soil and degree of infection by *Thielavia*.

⁴ Developed by James Johnson of the Wisconsin Agricultural Experiment Station.

TABLE 4.—*Effect of liming the soil on the growth of strains of tobacco resistant and susceptible to black root rot*

Strain of tobacco	Grown without lime			Grown with lime			Percent- age loss in yields on limed plots compared with yield on unlimed plots
	Soil pH value	Extent of root rot	Yield of tobacco per acre	Soil pH value	Extent of root rot	Yield of tobacco per acre	
			Pounds			Pounds	
Susceptible.....	5.4	Trace.....	1,294	6.0	Severe.....	821	37
Resistant, No. 142A3X.....	5.4	do.....	2,255	6.0	Trace.....	2,222	1
Resistant, No. 142C3X.....	5.4	do.....	2,176	6.0	do.....	2,049	6

The yield of the susceptible strain, with black root rot a factor, was 37 per cent less on limed than on unlimed plots. The yields of the resistant strains, with black root rot not a factor, were only 1 per cent and 6 per cent less on limed than on unlimed plots. In other words, when black root rot was not a factor, there was no real or significant decrease in yield as a result of the lime.

EFFECT OF SOIL REACTION ON GROWTH OF TOBACCO IN ABSENCE OF *THIELAVIA BASICOLA* COMPARED WITH ITS EFFECT IN THE PRESENCE OF THIS FUNGUS

Soils known to be infested with *Thielavia basicola* were obtained from a limed plot in which the pH value of the soil was 6.0, and from an adjacent unlimed plot in which the pH value was 5.5.

Half the soil obtained from each plot was steam sterilized to rid it of the fungus. As has been pointed out by others (10), an absolute comparison between sterilized and infested soils is subject to the criticism that changes in the soil in addition to the eradication of parasitic fungi result from steam sterilization. But methods of disinfecting soil without otherwise altering it are unknown, and for the purposes of this experiment, soil entirely free from *Thielavia basicola* was needed.

Five weeks after the soils were sterilized, tobacco seedlings (from sterilized soil) were set in pots of the several soils, with treatments in triplicate. The average dry weights per plant in each treatment at the end of the experiment are recorded in Table 5.

TABLE 5.—*Effect of liming the soil on the growth of tobacco with and without Thielavia basicola in the soil*

Description of soil	Average dry weight per plant grown
	Grams
Limed, <i>Thielavia</i> present.....	2.46
Not limed, <i>Thielavia</i> present.....	3.19
Limed, <i>Thielavia</i> absent.....	12.18
Not limed, <i>Thielavia</i> absent.....	3.45

Roots were examined. Infection by *Thielavia basicola* was severe in limed soil not sterilized, very mild or little more than a trace in soil not limed and not sterilized, and absent from sterilized soils limed and not limed.

When neither the limed nor the unlimed soil was sterilized, infection by the fungus in the limed soil so retarded the growth that plants in it had an average dry weight only 77 per cent of that of plants in unlimed soil.

When both these soils were sterilized, the benefit of lime was such that the average dry weight of plants in limed soils was 353 per cent of that of plants in soil without lime. In sterilized soil lime was associated with a marked increase rather than a decrease in the growth of tobacco plants.

In soil limed, and with the fungus consequently an important factor, the response in growth following the eradication of the fungus by steaming was an increase in weight of plants of 395 per cent. In soil not limed, and so without the fungus as an important factor, the response in growth following steaming was an increase in weight of plants of but 8 per cent, which is hardly significant. This may be interpreted to mean that a limed soil responded much more markedly to the stimulatory effect of steaming than did a soil without lime, but in any case it is evident that lime alone in the absence of *Thielavia basicola* did not injure the plants.

EFFECT OF SOIL TEMPERATURE ON THE RESPONSE OF TOBACCO TO CERTAIN SOIL REACTIONS IN THE ABSENCE OF THIELAVIA BASICOLA

The effect of soil reaction on the growth of tobacco in soil infested with *Thielavia basicola* is affected by the temperature of the soil. The experiments described below were undertaken to determine whether or not the response of tobacco to certain soil reactions is also affected by the temperature of the soil when this fungus is not a factor.

The soil used was freed of the fungus by steam sterilization. Its initial pH value was 5.6. The soil was divided into several portions and their reactions adjusted by means of hydrated lime, after preliminary experiments had shown the quantities of lime necessary to produce certain desired changes in the pH value of the soil. The quantities of lime used, on an acre basis, and the resulting pH values of the soil are shown in Table 6. This soil was placed in pots in constant temperature tanks, and one month after the application of lime, tobacco seedlings from sterilized soil were set in each pot.

TABLE 6.—Effect of soil temperature on the response of tobacco plants to lime in the absence of *Thielavia basicola*

Treatment of soil (per acre)	Soil pH value	Average dry weight (grams) per plant and percentage loss or gain in weight as a result of liming soil at a temperature of—											
		15° C.		18° C.		21° C.		24° C.		27° C.		30° C.	
		Weight	Loss or gain	Weight	Loss or gain	Weight	Loss or gain	Weight	Loss or gain	Weight	Loss or gain	Weight	Loss or gain
No lime.....	5.6	1.83	-----	4.33	-----	5.17	-----	4.69	-----	4.49	-----	4.08	-----
2.75 tons lime.....	6.0	1.04	-43	3.11	-28	4.99	-3	3.44	-27	6.04	+35	5.97	+46
5.5 tons lime.....	6.3	1.05	-43	2.37	-45	2.76	-47	6.42	+37	7.17	+59	6.49	+59
7.0 tons lime.....	6.5	.96	-48	2.00	-54	2.90	-44	3.91	-17	4.19	-7	6.78	+60

The average dry weights per plant at the end of the experiment in each treatment at each soil temperature are recorded in Table 6 together with the percentage increase or decrease in dry weights of plants in each lime treatment as compared with the weight of plants without lime at the same temperature. The effects of the several treatments on growth of plants are also shown in Figure 3.

In the sterilized soil, with *Thielavia* absent, plants grew markedly better without lime than with lime at the lower soil temperatures, below 24° C.; but plants grew markedly better with lime than without lime at the higher soil temperatures, 27° and 30° C. (Table 6.)

After the plants in this series were removed, tobacco seedlings were again set in the soil without lime and in the soil which received lime at the rate of 2.75 tons per acre. At the end of this experiment, the dry weights of plants in the soil with lime were, as compared with the dry weights of plants in soil without lime, as follows: 54 per cent less at 15° C., 59 per cent less at 18° C., 5 per cent less at 21° C., 11 per cent less at 24° C., 39 per cent greater at 27° C., and 87 per cent greater at 30° C. As in the preceding experiment, lime at the higher soil temperatures resulted in plants larger and at the lower soil temperatures in plants smaller than the plants without lime at the same temperatures.

In these experiments, the effect of lime in soil without *Thielavia basicola* present

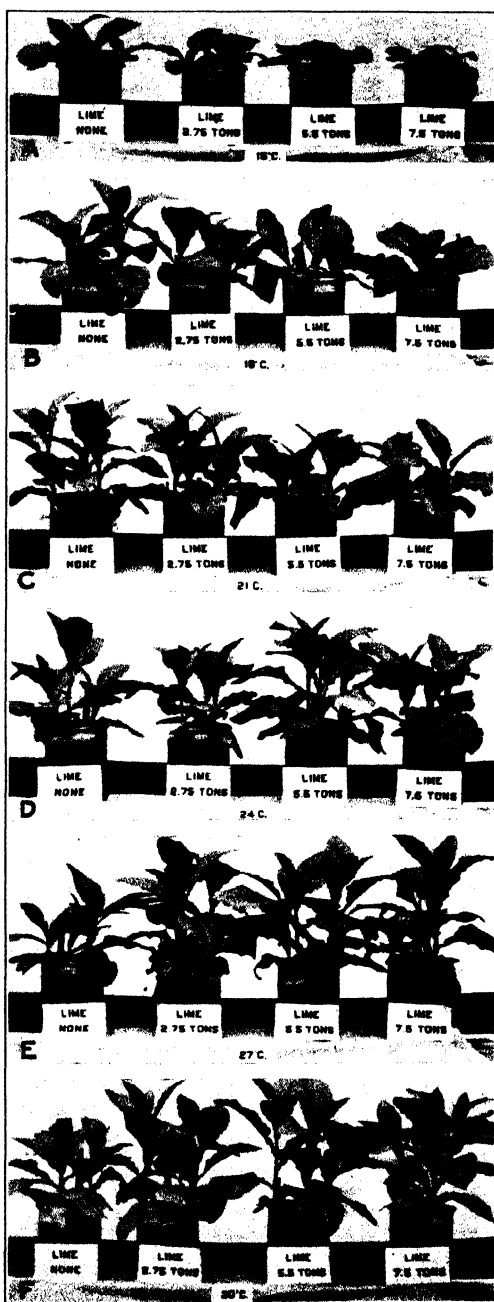


FIGURE 3.—Effect of soil temperature on the response of tobacco plants to lime in the absence of *Thielavia basicola*. Lime applications (per acre) and soil temperatures are indicated in the figure

was to increase growth of tobacco at soil temperatures optimum for its growth but to retard the growth of tobacco at lower temperatures. The effect of lime on tobacco in soil without this fungus was not unlike the effect which lime is known to exert on tobacco with *T. basicola* present. But if tobacco fails to grow at optimum soil temperatures in soils limed, even heavily limed, the cause is to be looked for in the blackroot-rot fungus rather than directly in the lime itself.

The response in the growth of tobacco to the beneficial effect of higher soil temperatures was greater with than without lime. (Table 6.) In this connection, results secured by Cerighelli (5) are of interest. He found that raising the temperature 10° , or to the optimum, increased the growth of roots of peas with calcium present but not without calcium.

No attempt is made to generalize from the results of these experiments, since the conditions were of course different from those in the field; the soil was steam sterilized, no nitrogenous fertilizers were applied, and relatively large amounts of lime were used to produce the desired changes in the pH value of the soil. It is not unlikely, however, that in the field, as in these pot experiments, an application of lime beneficial to tobacco at higher soil temperatures would retard the growth of this plant at lower soil temperatures. The explanation is probably to be found in the relation of lime to nitrification in the soil, for, although lime is favorable to nitrification, it is known that the decomposition of organic matter which results from liming may be accompanied by a loss of nitrogen from the soil if other conditions are unfavorable for the formation of nitrates, and one of these unfavorable conditions is known to be soil temperatures which are too low.

EFFECTS OF LIME, SOIL DISINFECTION, AND SOIL TEMPERATURE ON GROWTH OF TOBACCO PLANTS IN SEED BED

Since tobacco growers have learned the connection between lime and black root rot, few if any of them in this section now apply lime to the soil of the seed beds. It was probably applied more generally in the past, however, for Garner (8), describing in 1922 the culture of Connecticut Havana seed tobacco, mentions the application of lime, about 2 tons per acre, to tobacco seed beds (in the fall).

If lime benefits tobacco in the seed beds, it could of course be used without anticipating injury from black root rot in those seed beds in which the soil is sterilized. But the seed beds here are planted as early in the spring as the frost is out of the ground and when the soil temperatures are low. If the soil temperatures in the seed bed were for a long period low enough, injury rather than benefit from lime would be expected, even in sterilized soil, on the basis of the results of the experiments previously described in which lime interfered with the growth of tobacco at soil temperatures below 24° C., even in the absence of *Thielavia basicola*. The experiment now to be described was undertaken to secure further information as to the effect of lime on the growth of tobacco at known soil temperatures in the presence and in the absence of that fungus.

The soil in part of a seed bed was treated according to the standard method of soil disinfection with formaldehyde 1 to 50, 2 quarts per square foot being used. Two weeks later, one week before seeding, agricultural lime (50 per cent CaO) was applied at the rate of 2 tons

and of 4 tons per acre to plots in both the disinfected and the non-disinfected areas in this seed bed. Tobacco was seeded in all plots April 12, 1928.

By May 16, plants in formaldehyde-treated soil were larger than plants in soil without formaldehyde, but there was then no visible effect of lime. By May 31 the effect of lime on growth was evident. There was a conspicuous lag in the growth of plants on the formaldehyde-treated plot to which lime had been applied. The average dry weight of 100 typical plants removed from each plot on this date are recorded in Table 7, and the relative sizes of the plants at this time are shown in Figure 4.

TABLE 7.—*Effect of soil reaction on growth of tobacco in seed bed at known temperatures, with and without soil disinfection*

Treatment of soil (per acre)	Resulting pH value of soil ¹	Size of plants, May 31		Size of plants, June 14		Extent of black root rot
		Average dry weight	Relative weight ²	Average dry weight	Relative weight ²	
		<i>Grams</i>		<i>Grams</i>		
No disinfection; no lime.....	6.01	1.57	100	8.70	100	Moderate.
No disinfection; 2 tons lime.....	7.06	1.26	78	6.80	79	Severe.
Formaldehyde; no lime.....	5.96	5.53	348	6.10	70	Trace.
Formaldehyde; 4 tons lime.....	6.90	2.14	136	5.40	62	Mild.
Formaldehyde; 2 tons lime.....	7.00	2.50	159	5.60	64	Do.

¹ Determined electrometrically, May 12, by means of Youden hydrogen-ion concentration apparatus.

² Calculated with average dry weight of plants grown in unlimed, undisinfected soil as a base.

In the absence of lime, plants were more than three times as large in the formaldehyde-treated plot as in the plot without formaldehyde. In soil to which formaldehyde was not applied, plants were smaller with lime than without lime (relative numbers 78 and 100, respectively). But the harmful effect of lime was relatively much greater in soil disinfected with formaldehyde than in soil not so treated. In soil treated with formaldehyde the application of lime (2 tons per acre) resulted in plants less than half the size of plants without lime (dry weights expresses as relative numbers, 159 and 348, respectively).

On May 31 plants from the plot treated with formaldehyde but with no lime were large enough to transplant to the field; but plants from the plots not treated with formaldehyde or plants from the plots which received both formaldehyde and lime did not become large enough to transplant until two weeks later.

The benefit of formaldehyde, either in inhibiting parasites or in directly stimulating plant growth was marked up to the end of May, after which, with soil temperatures, especially the daily minima, higher, plants without formaldehyde grew relatively more rapidly. Plants were again removed from each plot two weeks later (on June 14), air-dried, and weighed. The weights are recorded in Table 7. The increases in dry weights at this time, as compared with the dry weights of the plants in each treatment two weeks earlier, were as follows: No disinfection, no lime, 454 per cent; no disinfection, 2 tons lime, 439 per cent; formaldehyde, no lime, 10 per cent; formaldehyde, 2 tons lime, 124 per cent; formaldehyde, 4 tons lime, 152 per cent.

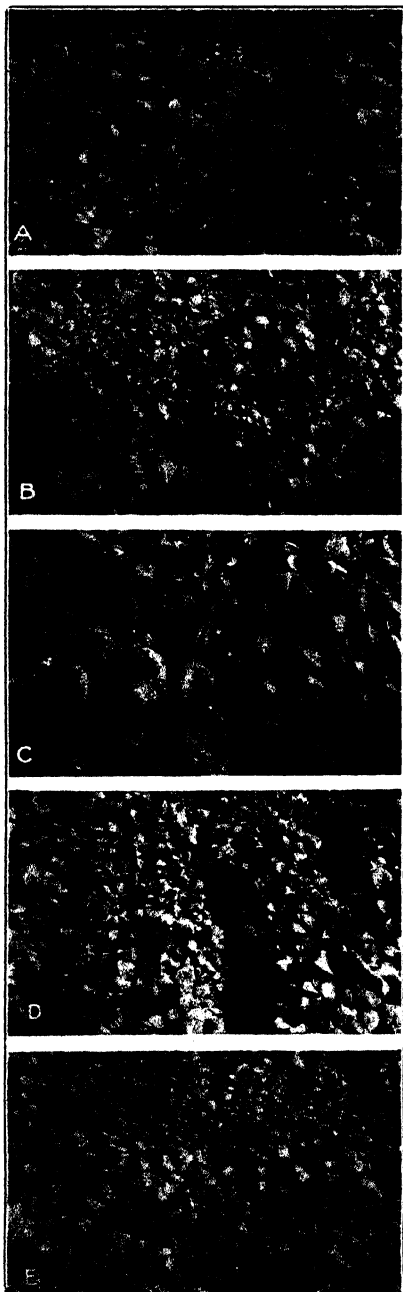


FIGURE 4.—Effects of lime on growth of tobacco in seed bed with and without soil disinfection with formaldehyde: A, No disinfection, no lime; B, no disinfection, 2 tons lime per acre; C, formaldehyde treatment, no lime; D, formaldehyde treatment, 2 tons lime per acre; E, formaldehyde treatment, 2 tons lime per acre

These differences resulted partly from the fact that the relatively rapid growth characteristic of tobacco plants at a certain age had already been made two weeks earlier by the plants in the formaldehyde-treated plot without lime.

Roots were examined June 14. In the plots without formaldehyde treatment, black root rot was a real factor; the disease was severe with lime and at least moderate without lime. There was some black root rot in all plots, even in those in which the soil received the formaldehyde treatment. However, there was no more than a trace of black root rot on plants in the plot with formaldehyde and no lime, and the disease was mild in formaldehyde-treated plots with lime. The formaldehyde was applied early in the spring, as is the usual practice at this station, and the soil was more or less saturated with water and packed. Under these conditions it is unlikely that formaldehyde treatment ever results in the complete absence of black root rot in a seed bed if the soil was infested before treatment.

A recording thermometer was placed in the seed bed and the temperature of the soil, at a depth of 2 inches, was thereby determined, beginning April 12. The daily maximum, minimum, and hourly mean temperatures so obtained are graphically expressed in Figure 5.

From April 12 to June 3, the hourly mean temperatures of the soil in the seed bed were always below 24° C., and the daily maximum temperatures of the soil were often below that point. The hourly mean temperatures of the soil in April were lower than they were later in the spring, although in this glass-covered seed bed the maximum daily temperatures were in some cases above 24° C. in April. But the daily ranges between maximum and minimum soil temperatures were greatest in April

because of the very low minimum soil temperatures in that month usually between 7° and 9° C.

This is the record in a cold and backward spring. (The mean hourly temperatures of the air were 2° C. below normal in April and 1.4° C. below normal in May at Amherst in 1928.)

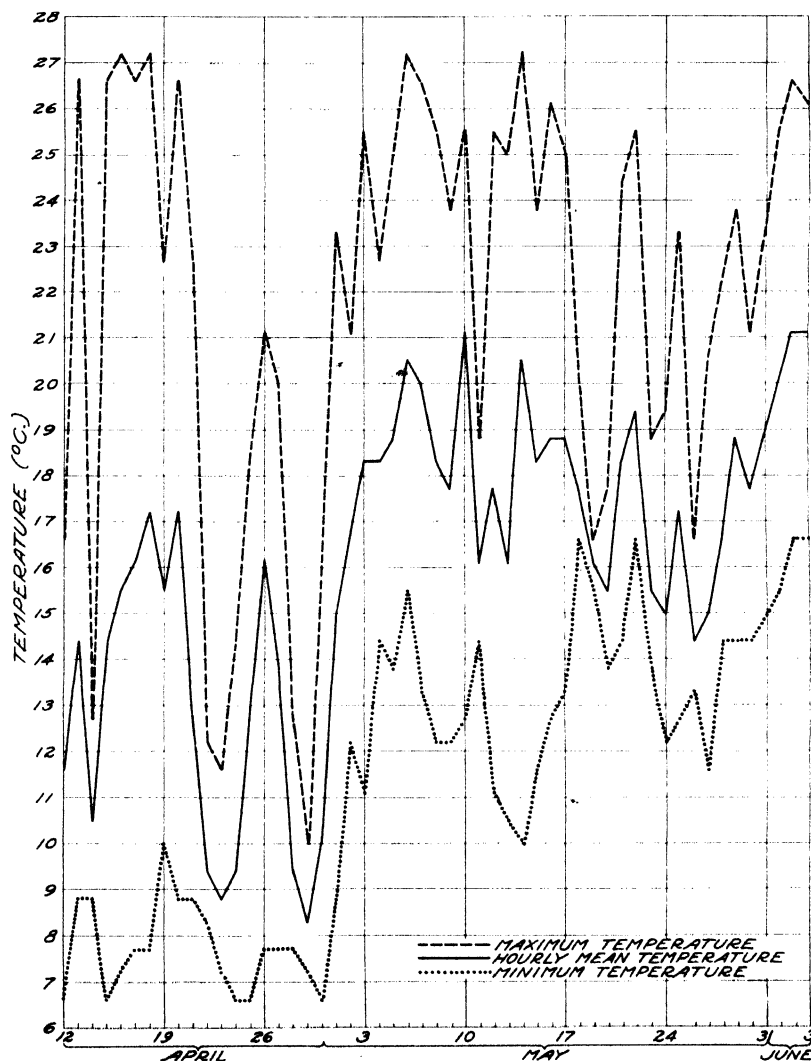


FIGURE 5.—Maximum, hourly mean, and minimum soil temperatures in tobacco seed bed, 1928

Under these conditions of low soil temperatures, the application of lime to the soil in the seed bed retarded the growth of tobacco, as it had in the pot experiments previously described, even when black root rot infection was reduced to no more than a trace by the use of formaldehyde. (It should be noted, however, that the disinfecting

effect of formaldehyde was not sufficiently complete to prevent some increase in black root rot when lime was applied to a formaldehyde-treated plot. It should further be noted that this soil was in no apparent need of lime for tobacco in the beginning, since it had an initial pH value of 5.96 to 6.01.)

But the evidence is unfavorable to the application of lime to a tobacco seed bed, at least in the spring, even though the soil has practically been rid of *Thielavia basicola* by formaldehyde, for in these experiments tobacco plants so treated were two weeks later in reaching transplanting size than were plants without lime, with the soil temperatures usually below 24° C.

In another experiment,⁵ lime was applied in the spring to the soil of a seed bed not sterilized. Growth of tobacco plants in this seed bed was not affected by applications of lime up to 1,600 pounds per acre. There was no black root rot on any of the plants, but growth was retarded by applications of lime at the rate of 2,000 pounds or more per acre.

SUMMARY

The critical point for black root rot of tobacco on the pH scale was affected somewhat by soil temperature. In the soil used there was no black root rot or only a trace at any temperature in soil with a pH value of 5.6 or lower. Marked injury began at pH 5.7 at 15° C., at pH 5.7 or 5.8 at 18° C., at pH 5.8 at 21° and 24° C., and at pH 5.8 or 5.9 at 27° C. There was little or no injury at 30° C., even in soil with pH values of 6.0 to 6.9.

Black root rot in the less acid soils at pH 5.9 to 6.5, with the soil temperature constant, retarded the growth of tobacco plants more when they were young than when they were older. Higher soil temperatures did not as effectively protect seedlings as they did older plants against retardation of growth caused by black root rot.

The germination of tobacco seeds was not affected by soil reaction within a range of soil pH values of 4.6 to 6.6. Soil temperatures between 15° and 30° C. had less effect on the percentage of seeds germinating than on the length of time required for germination.

The incubation period for infection of tobacco seedlings by *Thielavia basicola* was two to three weeks under optimum conditions. The colder the soil, down to 18° C., and the more nearly neutral the soil reaction (as compared to more acid soils) the shorter was the incubation period.

In the growing seasons of 1927 and 1928, the first characterized by slightly subnormal air temperatures with approximately normal precipitation and the second by normal air temperatures with excessive precipitation, the mean hourly temperatures of the soil in a tobacco field at Amherst, Mass., were below 24° C., and therefore favorable to black root rot in this soil (pH value about 6.0), with the exception of a few days in 1927. Under these conditions of soil temperature and soil reaction black root rot was sufficiently severe to reduce the yield of tobacco 40 per cent in 1927, as compared with the yield in the more acid soil of adjoining plots.

⁵ This experiment was conducted by J. P. Jones, of the Massachusetts station, who has kindly allowed the writer to use this material, not previously published.

In a soil heavily limed and consequently infested with *Thielavia basicola*, the yield of a strain of Havana tobacco susceptible to black root rot was 37 per cent less on limed plots than on unlimed plots, but the yield of strains resistant to black root rot was not significantly less on the limed plots than on the plots not limed, indicating that it is this fungus and not lime which is the direct cause of the poor growth.

In the absence of *Thielavia basicola*, the application of lime to a soil did not interfere with the growth of tobacco; on the contrary, it increased it.

The response in the growth of tobacco to certain soil reactions was affected by the temperature of the soil, even though *Thielavia basicola* was first eradicated by soil sterilization. Applications of lime which benefited tobacco at higher soil temperatures (27° and 30° C.) resulted in a retardation in its growth at lower soil temperatures (below 24° C.) in both cases as compared with the growth of tobacco without lime at the same temperatures.

In a glass-covered tobacco seed bed the hourly mean temperatures of the soil were below 24° C. from April 12 to June 3. Under these conditions the application of lime (by which the pH value of the soil was changed from 5.96 or 6.01 to 6.90 or 7.00) seriously retarded the growth of tobacco, even in soil in which black root-rot infection had been reduced to no more than a trace by the earlier formaldehyde treatment of the soil. The harmful effect of lime in the seed bed was relatively much greater in soil disinfected with formaldehyde than in soil not so treated.

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DEFICIENCY OF MAGNESIUM THE CAUSE OF A CHLOROSIS IN CORN¹

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INTRODUCTION

Many investigators have studied the chlorosis of plants and have found it to be induced by a number of conditions. If chlorosis, as defined by Clinton (3),² is "that unusual state of a green plant in which the chlorophyll, through either diseased or limited development, does not possess its normal bright green color, but becomes lighter, yellowish-green, or even distinctly yellowish or whitish," the types of chlorosis may be classified as follows: (1) Infectious chlorosis, such as the mosaics; (2) hereditary chlorosis, as shown in variegated plants; and (3) nutritional chlorosis, or a failure in the normal development of chlorophyll attributable to a specific nutrient deficiency or to a toxin.

The chlorosis with which this paper is concerned occurred on corn in one of the experimental fields at the Massachusetts Agricultural Experiment Station, known as North Corn Acre, and was first observed about 1920. It had probably been present prior to that time but in a less severe form, and so escaped attention. It was not until 1924 that definite work to determine the cause of chlorosis was undertaken. Observations up to this time showed (1) that no infectious disease was apparent, and (2) that the corn grown was inherently normal in color. It therefore appeared likely that the chlorosis was of the nutritional type. When the work was begun it was designed to attack the problem from the nutritional standpoint. This paper reports the results obtained.

DESCRIPTION OF THE CHLOROSIS

The symptoms of the chlorosis noted on North Corn Acre were manifested early in the season by a striping of the leaf. The striping was due to a differentiation in color of the intervascular and vascular tissues. About two weeks after the corn came up the intervascular tissue showed a light green color while the vascular tissue was a deep green, almost normal color. As the season progressed the light green color of the intervascular tissue continued to fade until all signs of green disappeared. Before the complete disappearance of the green color necrosis set in and the tissue turned yellow to yellowish brown and gradually ceased to function as a living part of the plant. The vascular tissues persisted in maintaining a green color almost until the adjacent tissues were dead, although evidences of fading appeared somewhat sooner. In extreme cases both the vascular and the intervascular tissues, particularly those near the border and tip of the leaf, lost their green color, turned yellowish

¹ Received for publication Apr. 11, 1929; issued December, 1929.

² Reference is made by number (italic) to "Literature cited," p. 891.

brown, and dried up prematurely. Illustrations of chlorosis at different stages may be seen in Figure 1.

Another symptom frequently noted was the bronzing and reddening of the leaves. In the early season this was observed chiefly near the margins but later it seemed to disappear. In the late

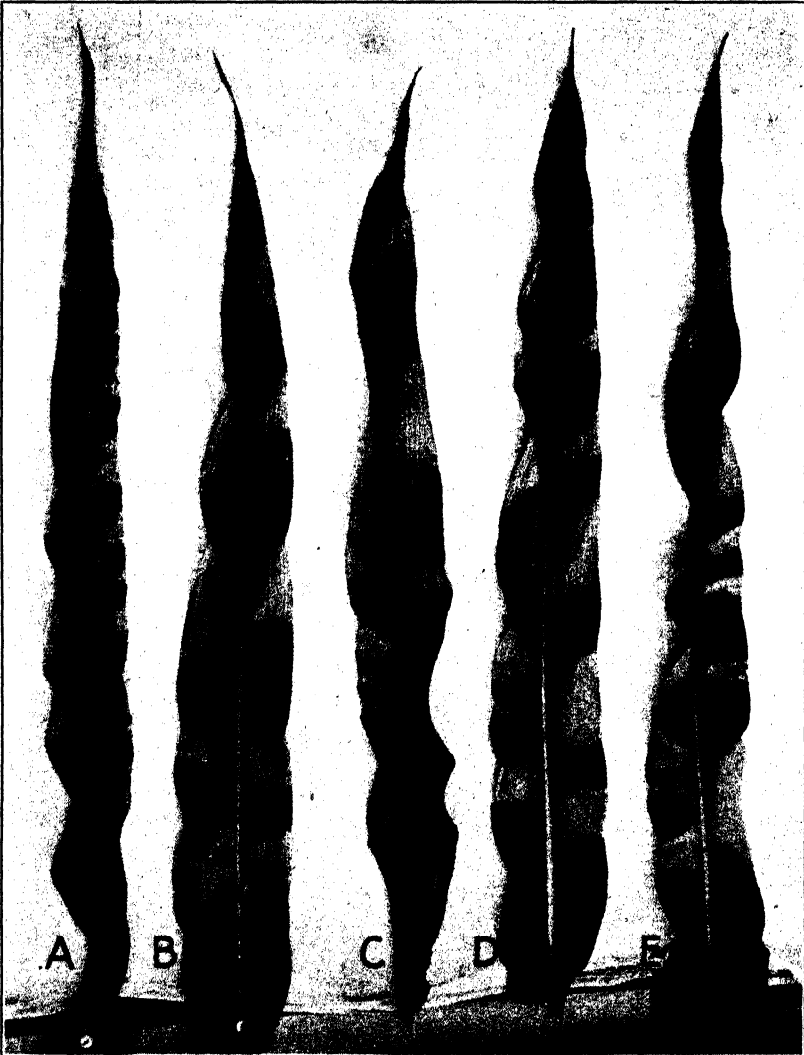


FIGURE 1.—Variation in degree of chlorosis on different corn leaves: A, Extreme chlorosis; B and C, medium chlorosis; D, slight chlorosis; E, normal green leaf

season, however, when the leaves began to mature, many deep-red leaves were observed. Whether this is a symptom closely associated with the chlorosis in question has not been definitely determined.

The chlorosis varied in severity among different plants and among leaves on the same plant. The bottom leaves seemed to be affected

most severely and the upper leaves only slightly. The tips and borders of the leaves were generally attacked most severely. The yellowing of the intervascular tissues in the early stages could usually be traced from the tip to the base of the leaf blades. The more severe aspects of the chlorosis, such as browning and dying of the tissue, were usually observed first in the regions near the tip and border of the leaf. It was also not uncommon to find plants exhibiting rather extreme cases of chlorosis growing within 3 feet of plants of a normal green color. The proportion of normal plants in a given area seemed to be greatest where the leaves showed only slight injury from chlorosis.

Whether the growth of the corn plant was affected seemed to depend upon the severity of the chlorosis. In light cases no visible differences in rate of growth were noticeable, but in extreme cases growth was reduced. The reduced growth was not especially marked early in the season, but in the latter part of the season the chlorotic corn grew somewhat more slowly and matured earlier. Even in cases where chlorosis was slight earlier maturity was characteristic.

CONDITIONS UNDER WHICH CHLOROSIS DEVELOPED

The field on which chlorosis developed had been used for fertilizer tests since 1891. Thus the conditions under which the chlorosis of the corn was brought about can be definitely described from the records.

The soil of North Corn Acre is a fine sandy loam underlaid by a gravelly fine sand which varies in depth from 12 to more than 40 inches. The field is well drained and has been classified as belonging to the Merrimac series. The topography is rolling, with a gently sloping ridge running diagonally from approximately northeast to southwest across the whole field. On this part a considerable amount of gravel is found in the surface soil.

During the period from 1891 to 1920, inclusive, two types of fertilizer for corn were being compared. One, commonly known as "special corn" fertilizer, was relatively high in phosphoric acid and low in potash; the other was relatively low in phosphoric acid and high in potash. For the purpose of this experiment North Corn Acre was divided into four plots, each one-fourth acre in size. Plots 1 and 3 received the special corn fertilizer and plots 2 and 4 received the fertilizer high in potash. The average annual application (1891 to 1920, inclusive) of nutrients furnished by the two fertilizers is shown in Table 1. Plot 4 received the same treatment as plot 2, except that from 1907 to 1918 inclusive an addition of 400 pounds of basic slag was applied annually, which accounts for the high average of P_2O_5 shown in Table 1 for plot 4.

The materials used to furnish the nutrients varied from time to time, the reason probably being the changes in the market supply that would normally occur during so long a period. As a source of nitrogen, nitrate of soda was used from 1891 to 1895 inclusive; and, with the exception of a small amount of sulphate of ammonia used in 1919 and 1920, nitrate of soda, dried blood, and fish were employed from 1896 to 1920 inclusive. The phosphoric acid was furnished by dissolved bone black from 1891 to 1895 inclusive, and by superphosphate (acid phosphate) and fish from 1896 to 1920 inclusive. Except in 1919

and 1920, when Nebraska potash was used, muriate of potash furnished the potash for the entire period from 1891 to 1920 inclusive. In spite of these variations in materials, the original purpose of comparing the two types of fertilizer was adhered to until 1921.

TABLE 1.—Quantity of nutrients and fertilizers applied annually to the different plots in North Corn Acre from 1891 to 1928, inclusive

Plot No.	Nutrients applied 1891 to 1920, inclusive, expressed in average pounds per acre			Fertilizers applied 1921 to 1928 expressed in average pounds per acre			
	NH ₃	P ₂ O ₅	K ₂ O	Nitrate of soda	Fish	Superphosphate (acid phosphate)	Muriate of potash
1.....	50	156	58	120	360		150
2.....	50	56	120	200	200	200	250
3.....	50	156	58	120	360	1,092	150
4.....	50	81	120	200	200	200	

Except in 1891 and 1892, when millet was planted on plots 3 and 4, corn was grown on all plots from 1891 to 1896, inclusive. In 1897 the cropping system was changed to two successive years of hay—a mixture of timothy, redbud, and clover—and then two successive years of corn. The hay mixture was seeded the second year in the corn. This cropping system has been used without change up to the present time.

No manure was applied during the entire period of the tests. Lime was used in 1900 at the rate of 1 ton per acre of air-slaked lime, in 1907 at the rate of 1 ton per acre of agricultural lime, and in 1921 at the rate of 2 tons per acre of ground limestone, but only on the north half of the plots.

The comparison between the two types of fertilizer revealed very little difference in response as measured by the yields of corn and hay. The average yields per acre of the crops grown up to 1921 were, for plots 1 and 3, 63.88 bushels of corn and 3,993 pounds of hay; for plots 2 and 4, 62.20 bushels of corn and 4,229 pounds of hay. These yields are based on field weights rather than on a dry basis. In view of the variability of the data, little significance can be attached to these differences in yields. Part of this variability is believed to have been due to the manner in which variation in rainfall affects the different plots. During a dry season crops on the section of the plots through which the ridge runs suffer severely, the different plots being unequally affected. Probably in some seasons the environmental variation was of greater significance than the difference in treatments.

HYPOTHESES TO ACCOUNT FOR DEVELOPMENT OF CHLOROSIS

In considering the cause of the chlorosis from the nutritional standpoint, two factors seemed to deserve first consideration: (1) The presence in the soil of an excess of soluble aluminum and (2) the lack of magnesium.

ALUMINUM TOXICITY

Preliminary observations showed that the corn growing on the half of the field limed in 1921 was less severely affected by chlorosis than that on the unlimed half. This pointed definitely to the con-

clusion that lime was capable of overcoming chlorosis, and furnished the basis for suspecting that aluminum toxicity was involved.

When Abbott, Connor, and Smalley (1) first noted poor crop yields associated with an excess of soluble aluminum salts they found applications of pulverized limestone under field conditions to be an effective remedy. The later work of Hartwell and Pember (5), Mirasol (11), and Burgess and Pember (2) showed superphosphate to have a capacity similar to that of lime in counteracting the depressing effects of aluminum. Their work indicated that for practical purposes moderate applications of lime and superphosphate were more effective than either alone in correcting the acidity of soils containing large amounts of active aluminum. If aluminum were a factor in causing the chlorosis on North Corn Acre, these results suggest that the chlorosis should have been less severe on plots 1 and 3, which received the high applications of superphosphate. (Table 1.) Preliminary observations, however, did not indicate any lessening of the chlorosis associated with the high superphosphate treatments.

Burgess and Pember (2) have classified certain crop plants according to their resistance to aluminum toxicity. In their classification corn is rated as very resistant, being capable of normal growth on soil having a pH value below 5.3 and an active aluminum content above 500 parts per million of dry soil. Magistad (10), working with nutrient solutions, showed that toxicity of aluminum to the corn plant varied with the pH, at pH 4 the toxic effect was very severe, while at pH 6.4 it was slight. The preliminary field observations indicated that the chlorosis appeared on soil having a pH value between 5 and 5.4.

Hoffer (6) has reported that aluminum salts are especially effective in plugging the vascular bundles of the corn plant. He also described the manner in which aluminum affected the foliage. The leaves first showed a slight yellowing of the intervascular tissue, resulting in a yellow streaked appearance, then a water-soaked effect, and finally in necrosis. These observations are quite similar to those recorded for the corn leaves on North Corn Acre. However, there was no evidence that the nodal tissue of the corn was destroyed as Hoffer (6) observed in cases of severe aluminum injury.

MAGNESIUM DEFICIENCY

Magnesium, being a constituent of chlorophyll and necessary for normal green color in plants, was suspected of being deficient in the soil on North Corn Acre and therefore a possible cause of the chlorosis. Garner and his associates (4) found that sand drown, a chlorosis of tobacco, resulted from a deficiency of magnesium in certain soils of the South and of the Connecticut Valley. They stated that applications of cottonseed meal, barnyard manure, tobacco stalks, and tobacco stems were effective in controlling sand drown. The effectiveness of these materials was explained as due to the magnesium which they contained. As mentioned above, the fertilizers used on North Corn Acre were practically free of magnesium. No barnyard manure or vegetable organic fertilizer has been applied in 37 years. The finding of sand drown on tobacco in the Connecticut Valley, in spite of the fact that it has been the practice to fertilize to a limited extent with barnyard manure and vegetable organic materials for many years, strongly supported the suspicion

that a magnesium deficiency existed on North Corn Acre. This evidence seemed particularly suggestive when it was recognized that the soil on this field is quite similar to that on which much of the tobacco is grown.

On North Corn Acre, as noted above, lime was effective in reducing chlorosis. The records showed that the lime used contained about 5 per cent magnesium oxide. Garner and his coworkers claim that liming the soil even with fairly pure limestone should prevent the sand-drown type of chlorosis. In field experiments in North Carolina (8) the yield and quality of tobacco have been improved by treating the soil with magnesian limestone.

METHOD OF PROCEDURE

The method of procedure was developed to determine whether lime prevented chlorosis through counteracting soluble aluminum or through furnishing needed magnesium. Field work was supplemented by greenhouse and chemical studies.

FIELD STUDIES

RESIDUAL VALUE OF PHOSPHORIC ACID AND POTASH IN THE SOIL

In 1921 the old objective of the North Corn Acre experiment—to compare two different fertilizers for corn—was abandoned. In its place was substituted an attempt to observe the residual value of the previous high applications of phosphoric acid and potash. This was done by omitting superphosphate from the fertilizer applied to plot 1. The residual influence of the superphosphate was expected to be shown by a comparison of plot 1 with plot 3, on which the original high applications of superphosphate were continued. In like manner potash was omitted from the fertilizer treatment on plot 4 with the expectation that a comparison with the continued high applications on plot 2 would show the residual effects of the many years of high potash treatment. The annual fertilizer applications from 1921 to 1928, inclusive, are shown in Table 1.

In Table 2 have been included data bearing on the residual value of the long treatment with both high potash and superphosphate. No yield records are given for 1928 because wireworm injury on certain areas so affected the yields that they can not be considered to represent the effects of the treatments reliably. The records for hay were also omitted because hay was grown in 1921 and 1922 immediately after the omission of superphosphate and potash from the treatments of plots 1 and 4, and no evidence of a shortage of these fertilizers was noted. In 1925 and 1926, when hay again occupied the field, the dry weather during the growing season affected the growth to such an extent that no yield records were taken.

It is apparent from Table 2 that the omission of the superphosphate from the fertilizer treatment on plot 1 did not affect the yield of either the corn or the stover. This may be taken to indicate that phosphoric acid (P_2O_5) applied at the rate of about 156 pounds per acre annually for 30 years in a rotation of corn and hay builds up a substantial residuum in the soil. On this field it was sufficient to maintain the yield of corn and stover for at least 7 years.

On the other hand, Table 2 reveals that an application of 120 pounds of potash (K_2O) per acre for 30 years did not permit a very large accumulation. However, the accumulation was sufficient to maintain the yield of corn on plot 4 the third year after the potash was left out of the fertilizer application. In the fourth and seventh years plot 4 as compared with plot 2 where the original high potash treatment was continued showed a decline in yield of corn of about 13 per cent. No consistent decline in yield of stover was apparent.

TABLE 2.—*Residual effect of phosphorus and potash on the yield per acre of corn and stover*

[Dry weight basis]

Plot No.	Yield in 1923		Yield in 1924		Yield in 1927		Average yield	
	Corn		Corn		Corn		Corn	
	Bushels	Pounds	Bushels	Pounds	Bushels	Pounds	Bushels	Pounds
1.....	35.0	2,366	34.6	1,785	40.2	2,026	36.6	2,059
3.....	36.9	2,092	33.2	2,184	43.2	1,921	37.8	2,066
2.....	30.3	2,389	31.1	1,653	36.8	1,861	32.7	1,968
4.....	32.5	2,044	27.1	1,738	32.1	1,681	30.6	1,821

EFFECT OF LIME AND MAGNESIUM ON THE DEVELOPMENT OF CHLOROSIS

Cross treatments of the plots on which the residual effects of phosphoric acid and potash were being studied were made to compare the influence of magnesium and lime on the chlorosis of corn. Figure 2 shows the plan of the field. The numbers employed refer to the plots as they were laid out in 1891 and the letters to the cross treatments. Magnesium sulphate was applied in the spring of 1924 and 1927 at the rate of 200 pounds per acre and in 1928 at the rate of 400 pounds per acre. This gave cross treatments as follows: Section A, 2 tons of ground limestone per acre applied in 1921; section B, 2 tons of ground limestone applied in 1921 plus magnesium sulphate at the rates noted above; section C, magnesium sulphate at the same rate as on section B; section D, check, no lime or magnesium sulphate.

To determine the effects of the cross treatments on chlorosis, counts were made of what were recognized in the field as normal hills and as slightly, medially, and extremely chlorotic hills. For the normal hills only those entirely free of the typical chlorosis were counted. The attempt to classify the chlorosis into slight, medium, and extreme cases was based largely upon the judgment of the observer, and the line of demarcation between these groups can not be considered as accurate as that between the normal and chlorotic groups. The classification does, however, afford a basis for estimating the severity of the chlorosis.

In Table 3 the observations for 1924, 1927, and 1928 are recorded. Considering first the results for 1924, it will be noted that practically no chlorosis occurred on sections A and B on any of the plots. Such as did occur was rated as slight and probably would not be noticed under practical conditions. It will be recalled that A and B both received lime in 1921 and B magnesium sulphate in 1924. These records are in accord with the preliminary observations which showed that chlorosis was not very apparent on the limed area.

On section C, which received the magnesium sulphate treatment, the proportion of normal hills was somewhat smaller than on the limed area. Except on plot 2, where 12.38 per cent of medially chlorotic hills was noted, the chlorosis was rated as slight. In comparison with section D, which received neither lime nor magnesium sulphate, the treatment on section C seems to have been very effective. This is manifested both by the greater proportion of normal hills on each plot and by the milder form of the chlorosis.

The magnesium sulphate treatment was made about 10 days before the corn was planted. Magnesium sulphate may not have been applied

in a quantity large enough or it may not have become sufficiently mixed through the soil to supply the amount of magnesium needed to completely overcome the chlorosis. It is a common observation that magnesium sulphate applied to soil persists in coming to the surface. Thus, not enough may have been available to the roots in all cases. The evidence for 1924, however, indicated that the magnesium sulphate performed a function similar to that of lime in tending to eliminate the chlorosis.

4 A	3A	2A	1A
4B	3B	2B	1B
4C	3C	2C	1C
4D	3D	2D	1D

FIGURE 2.—Plan of North Corn Acre, the field used for the study of chlorosis; dimensions 214 by 203.5 feet; size of plots one-sixteenth acre. Sections A and B were limed in 1921 at the rate of 2 tons per acre; sections B and C had $MgSO_4$ applied at the rate of 200 pounds per acre in 1924 and 1927, and at the rate of 400 pounds per acre in 1928; section D received neither lime nor $MgSO_4$. The fertilizer treatment of plots 1, 2, 3, and 4 is shown in Table 1

TABLE 3.—Percentage of normal and chlorotic corn hills in the various plots of North Corn Acre in 1924, 1927, and 1928

Plot No.	1924				1927				1928			
	Normal hills	Chlorotic hills			Normal hills	Chlorotic hills			Normal hills	Chlorotic hills		
		Slightly	Medially	Extremely		Slightly	Medially	Extremely		Slightly	Medially	Extremely
1A	99.56	0.44	0	0	98.33	1.67	0	0	77.40	22.60	0	0
1B	99.52	0.48	0	0	97.92	2.08	0	0	97.12	2.88	0	0
1C	80.86	19.14	0	0	84.85	15.42	0	0	97.12	2.88	0	0
1D	31.43	48.57	17.62	2.38	14.17	38.33	31.67	15.83	6.25	16.83	27.88	49.04
2A	98.66	1.34	0	0	92.92	7.08	0	0	75.38	21.61	3.01	0
2B	99.05	.95	0	0	97.08	2.92	0	0	98.50	1.50	0	0
2C	51.91	35.71	12.38	0	82.77	17.23	0	0	98.08	1.92	0	0
2D	11.49	22.01	45.45	21.05	2.97	12.29	26.27	58.47	5.80	16.91	29.95	47.34
3A	99.55	.45	0	0	94.12	5.88	0	0	82.21	16.83	.96	0
3B	98.55	1.45	0	0	96.25	3.75	0	0	94.23	5.77	0	0
3C	86.12	13.88	0	0	92.89	7.11	0	0	98.56	1.44	0	0
3D	17.62	50.48	30.47	1.43	32.08	58.33	9.17	.42	6.76	38.65	42.51	12.08
4A	96.64	3.36	0	0	97.91	2.09	0	0	96.15	3.85	0	0
4B	95.11	4.89	0	0	99.17	.83	0	0	96.64	3.36	0	0
4C	90.22	9.78	0	0	97.08	2.92	0	0	97.09	2.91	0	0
4D	78.12	20.54	1.34	0	30.12	54.81	14.23	.84	20.67	36.95	13.79	28.57

After the 1924 experiments corn was not again grown until 1927. In 1925 and 1926, when hay was grown, no magnesium was applied. The observations on chlorosis made in 1927 are shown in Table 3. The treatments with lime alone and with a combination of lime and magnesium sulphate were both effective in controlling chlorosis. On plots 3C and 4C the magnesium sulphate treatment was practically as effective as where it was applied in the limed area. On all the plots magnesium sulphate controlled chlorosis better than in 1924. This was evidenced by a greater percentage of normal hills and a less severe type of chlorosis. On the other hand, the chlorosis on section D, with the exception of that on plot 3D, was more prevalent in 1927 than in 1924 and was also more severe.

Corn was grown again in 1928, and the effects of the various treatments on chlorosis are shown in Table 3. It should be recalled at this point that 400 pounds of magnesium sulphate per acre were applied to sections B and C. Magnesium sulphate practically eliminated the chlorosis, a fact outstanding in the table. It was fully as effective when applied alone as when added to the limed area, and was more effective than lime alone. In 1928 the percentage of normal hills on section A was smaller than in 1924 and 1927. This may mean that the lime was no longer as effective in controlling the chlorosis as it was in the earlier years following its application. On section D, with the exception of plot 2D, where chlorosis was about as bad, there were fewer normal hills and a larger proportion of extremely chlorotic hills in 1928 than in 1927.

In 1928 two rows of tobacco were planted with the corn through each plot to check further the magnesium hypothesis. This was done because the writer was not familiar with the symptoms of magnesium hunger on corn but was somewhat acquainted with them on tobacco, and it was thought that the behavior of the tobacco, with reference to the cross treatments which had proved successful in controlling chlorosis of corn, might afford a means of distinguishing between the effects of the lime and magnesium sulphate.

On section D of every plot typical sand drown was noted on many of the tobacco plants within two weeks after they were set. It is unusual to find this type of chlorosis so early in the season, particularly when it is considered that the fertilizer used for the tobacco carried cottonseed meal in quantity equivalent to about 1,100 pounds per acre. Garner and his associates (4) had found in their experiments that about half this amount of cottonseed meal decidedly reduced sand drown. The tobacco on section D, however, showed a very severe case of sand drown, and by the end of the season scarcely a leaf on any of the plants in the entire section was free of it.

Sand drown of tobacco and chlorosis of corn both responded to the same treatments. On sections B and C, where the chlorosis was reduced to a minimum by the magnesium sulphate treatment, the tobacco showed no evidence of sand drown. The line of demarcation between the plots on sections C and D could easily be distinguished by the absence of sand drown and chlorosis in the one case and their presence in the other. On section A there was evidence, similar to that for chlorosis on the corn, that the lime was not controlling the sand drown. Table 4 shows that 28.08 per cent of the tobacco plants were affected by sand drown.

If the records of Table 4 are compared with those of Table 3 it is evident that a correlation exists between the effect of magnesium sulphate on chlorosis of corn and on sand drown of tobacco. Since sand drown has already been proved to be due to a deficiency of magnesium, this correlation suggests very strongly that the lack of magnesium is the cause of chlorosis on the corn.

TABLE 4.—Percentage of tobacco plants free of and affected by sand drown when planted on North Corn Acre in 1928

Plot	Normal plants	Plants affected by sand drown		Plot	Normal plants	Plants affected by sand drown	
		Medially	Extremely			Medially	Extremely
1A.....	54.39	45.61	0	3A.....	63.16	36.84	0
B.....	100.00	0	0	B.....	100.00	0	0
C.....	100.00	0	0	C.....	100.00	0	0
D.....	3.39	8.48	88.13	D.....	0	12.73	87.27
2A.....	94.64	5.36	0	4A.....	75.47	24.53	0
B.....	100.00	0	0	B.....	100.00	0	0
C.....	100.00	0	0	C.....	100.00	0	0
D.....	11.86	37.29	50.85	D.....	0	12.73	87.27

Table 5 gives a summary showing the variation by years in the total percentage of chlorosis. The severity of the disease is disregarded here, but the figures accurately represent the proportion of the corn affected. On section D the tendency has been for the chlorosis to increase each year. On section C, where magnesium sulphate was added, the tendency has been just the reverse, the smallest percentage of chlorotic hills occurring the third year, 1928. Where both lime and magnesium sulphate were used (section B) chlorosis was controlled about equally each year. On section A, where lime alone was used, chlorosis was held in check in 1924 and 1927, but in 1928 there was evidence that the effectiveness of the lime was much reduced.

TABLE 5.—Percentage of chlorotic hills of corn occurring in the various plots of North Corn Acre during different years

Plot	Chlorotic hills occurring in—			Average	Plot	Chlorotic hills occurring in—			Average
	1924	1927	1928			1924	1927	1928	
1A.....	0.44	1.67	22.60	8.24	3A.....	.45	5.88	17.79	8.04
B.....	.48	2.08	2.88	1.81	B.....	1.45	3.75	5.77	3.66
C.....	19.14	15.42	2.88	12.48	C.....	13.88	7.11	1.44	7.48
D.....	68.57	85.83	93.75	82.72	D.....	82.38	67.92	93.24	81.18
2A.....	1.34	7.08	24.62	11.01	4A.....	3.36	2.09	3.85	3.10
B.....	.95	2.92	1.50	1.79	B.....	4.89	.83	3.36	3.03
C.....	48.09	17.23	1.92	22.41	C.....	9.78	2.92	2.91	5.20
D.....	88.51	97.08	94.20	93.26	D.....	21.88	69.88	79.31	57.02

These variations in chlorosis with the different seasons are interesting in connection with the precipitation records shown in Table 6. It has been a common observation that, when fertilization is the same, sand drown is more prevalent in seasons of excessive rainfall than in seasons of light rainfall. Table 6 shows that the rainfall for the growing seasons of 1927 and 1928 was about two and three times, re-

spectively, that of 1924. It also shows that about 27 per cent of the total rainfall occurred during the growing season of 1924, 35 per cent during that of 1927, and 59 per cent during that of 1928.

TABLE 6.—*Inches of rainfall during the growing season on North Corn Acre in 1924, 1927, and 1928*

Month	Rainfall in—		
	1924	1927	1928
May.....	2.21	4.83	3.25
June.....	1.28	3.37	6.97
July.....	1.75	3.40	6.23
August.....	3.11	5.01	8.40
Total.....	8.35	16.61	24.85
Total precipitation for the year.....	30.96	46.96	42.06

Chlorosis on section D, where no cross treatment of magnesium was made, increased each year. This increase is correlated with the increase in rainfall. In 1928, when the rainfall was heaviest, the chlorosis was most severe. Observations on tobacco grown in the Connecticut Valley showed practically no sand drown in 1924, somewhat more in 1927, while in 1928 it could be found in almost every field. Garner and his associates (4) also recognized that sand drown is more widespread in seasons of heavy rainfall. It therefore seems that a relation exists between the manner in which excessive rainfall during the growing season affects chlorosis and the manner in which it affects sand drown. This relation would seem to indicate that the chlorosis was caused by the same deficiency as sand drown.

EFFECT OF DIFFERENT FERTILIZERS ON THE DEVELOPMENT OF CHLOROSIS

Reference to Table 1 will show the annual fertilizer treatments received by the different plots from 1921 to 1928, inclusive. Prior to 1921 plots 1 and 3 had been duplicates, but since 1921 the high phosphoric acid treatment has been discontinued on plot 1. Until 1921 the only difference between the fertilizer treatment of plots 2 and 4 was that plot 4 received annually, from 1907 to 1918 inclusive, 400 pounds of basic slag per acre, but since 1921 the high potash has been omitted from the treatment of plot 4.

On section D, where there has been no cross treatment, it is possible to compare the influence of the fertilizers on the development of chlorosis. Table 7 shows the relative amounts of chlorosis in 1924, 1927, and 1928 by plots for section D. The outstanding fact revealed by this table is that chlorosis was present on all plots regardless of the fertilizer used. On plot 1D, where phosphorus was omitted, there was apparently less chlorosis in 1924 than on plot 3D, where the original high phosphorus treatments were continued, but in 1927 the reverse was true. In 1928 these two plots had about the same amount of chlorosis. Plot 2D, where the high potash treatment was continued has shown consistently a larger proportion of chlorotic hills than plot 4D, where the potash treatment was discontinued. In the data presented above it was shown that both magnesium and lime treatments were curatives for the chlorosis. The basic slag used on plot

4 for 12 years carried both lime and magnesium. This may account for the reduced amount of chlorosis on plot 4D as compared with that on other plots. So far as plots 1, 2, and 3 are concerned, there is not enough consistency in the data to show that the different fertilizers used have influenced the proportion of chlorotic hills. In 1928, when conditions were most favorable for chlorosis, these three plots were almost identical so far as the number of chlorotic hills was concerned.

Except in plot 4, where some magnesium and lime were added in the form of basic slag, it can be said that the development of chlorosis was independent of the fertilizers used. If, as suspected at the beginning of the experiment, chlorosis was due to aluminum toxicity the proportion of chlorotic hills should have been reduced by the high applications of phosphorus; but since the amount of chlorosis seemed to be about as large on the high phosphorus as on the other plots the aluminum hypothesis appears untenable.

TABLE 7.—*Influence of different fertilizer treatments on the chlorosis of corn in section D of North Corn Acre*

Plot	Chlorotic hills occurring in—			Average	Plot	Chlorotic hills occurring in—			Average
	1924	1927	1928			1924	1927	1928	
	<i>Per cent</i>	<i>Per cent</i>	<i>Percent</i>	<i>Per cent</i>		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1D.....	68.57	85.83	93.75	82.72	3D.....	82.38	67.92	93.24	81.18
2D.....	88.51	97.03	94.20	93.25	4D.....	21.88	69.88	79.31	57.02

EFFECT OF CHLOROSIS ON YIELD

In Table 8 are presented figures which show the effect of chlorosis on the yield of corn. In compiling the figures four plots were averaged in each case to obtain the yield per acre for the different sections. In 1928, however, because of wireworm injury on certain sections of the field, only the yield records for those plots where the injury was slight were included. Even with this precaution the yields indicate that the amount of replanting made necessary by the wireworm injury, particularly on sections B and C, should be considered. Except in 1928, sections A, B, and C are very close in yield of both corn and stover. Section D each year has consistently yielded less of both corn and stover than the other sections. Considering the average yield of corn for sections A, B, and C as compared with that for section D, they outyielded the latter by about 21 per cent in 1924, 6 per cent in 1927, and 34 per cent in 1928. If stover is considered similarly, the differences are 7 per cent for 1924, 4 per cent for 1927, and 16 per cent for 1928.

The average percentage of chlorotic hills for sections A, B, and C varied from 2.57 to 11.89. It should be recalled that section A received the lime treatment, section B the lime plus magnesium sulphate, section C the magnesium sulphate alone, and that section D was reserved as a check. It is evident from Table 8 that the chlorosis did not appreciably affect the yield of corn or stover when the chlorosis was controlled as it was on sections A, B, and C. On section D, however, where the average percentage of chlorotic hills reached

78.57 per cent, there was a depression in yield. The corn yields appeared to be more severely affected than the stover yields. These data suggest that chlorosis, though manifesting itself mostly on the foliage of the corn plant, does not affect the growth of the plant as much as might be expected. The major effect of chlorosis was on the production of seed.

TABLE 8.—*Effect of chlorosis on the yield per acre of grain and stover*

[Dry weight basis]

Section	Yield in—						Average		Chloro- tic hills
	1924		1927		1928				
	Corn	Stover	Corn	Stover	Corn	Stover	Corn	Stover	
	<i>Bushels</i>	<i>Pounds</i>	<i>Bushels</i>	<i>Pounds</i>	<i>Bushels</i>	<i>Pounds</i>	<i>Bushels</i>	<i>Pounds</i>	
A.....	33.5	1,804	38.5	1,901	37.0	2,112	36.3	1,959	7.60
B.....	33.9	1,945	37.7	1,785	32.5	1,879	34.7	1,870	2.57
C.....	31.4	1,826	39.6	1,980	31.4	1,828	34.1	1,878	11.89
D.....	27.2	1,749	36.4	1,820	25.0	1,676	29.5	1,748	78.54

GREENHOUSE STUDIES

In the above discussion of field work it was evident that both lime and magnesium sulphate possessed the ability to control the chlorosis. In the greenhouse, an attempt was made to determine whether the effect of the lime was due to its ability to counteract aluminum toxicity or to the magnesium it contained. Soil was taken from the section of the field showing the highest percentage of chlorotic corn hills, placed in pots, and fertilized uniformly with a complete fertilizer free of magnesium. The fertilizer applications and the treatments shown in Table 9 were made on January 12 and the corn was planted on January 26. The soil was maintained at a uniform moisture content while the corn was growing. The corn was thinned to two stalks per pot. On April 18 the crop was harvested.

TABLE 9.—*Influence of different fertilizer treatments on the yield and extent of chlorosis of corn grown in the greenhouse*

Pot No.	Treatment per acre	Yield of dry matter per pot	Extent of chlorosis
		<i>Grams</i>	
1-4.....	400 pounds $MgSO_4 \cdot 7H_2O$	40.48	None.
5-8.....	800 pounds $MgSO_4 \cdot 7H_2O$	38.49	Do
9-12.....	1,200 pounds $MgSO_4 \cdot 7H_2O$	33.10	Do
13-16.....	Check.....	29.15	Extreme.
17-20.....	2 tons agricultural lime *.....	30.15	Slight.
21-24.....	2 tons C. P. $CaCO_3$	23.83	Extreme.
25-28.....	1,000 pounds superphosphate.....	33.11	Do.
29-32.....	2,000 pounds superphosphate.....	32.49	Do.

* Contained 5 per cent MgO .

In Table 9 are recorded the yield of dry matter per pot and notes on the appearance of the corn with reference to chlorosis. Where magnesium sulphate at the rate of 400 and 800 pounds per acre was

used the yields were highest and the corn was free of chlorosis. Where magnesium sulphate at the rate of 1,200 pounds per acre was used the yield was not quite so good as where the smaller amounts were applied, but no chlorosis was noted. The smallest yields were obtained on the check pots and on those receiving the chemically pure calcium carbonate. The yield from the pots that received agricultural lime was very similar to that from the pots that received superphosphate. However, with only four replications the differences are in most cases too small to interpret with confidence.

The effect of the treatments on chlorosis were much more clear cut than on the yields. The first notable feature was that both magnesium sulphate and agricultural lime counteracted the chlorosis just as they did in the field. That a slight amount of chlorosis appeared



FIGURE 3.—The comparative effect of magnesium sulphate and superphosphate as preventives of chlorosis in corn grown on soil from fields which produced chlorotic corn: A, Magnesium sulphate was applied to the soil in this pot at the rate of 400 pounds to the acre; B, superphosphate was applied at the rate of 2 tons per acre

where the agricultural lime was used was attributed to the fact that the lime had had only about 14 days in which to become thoroughly mixed in the soil before the corn was planted. This was probably not long enough for the magnesium in the lime to become adequately available. Where the chemically pure lime (fig. 4) was used chlorosis was extreme. It was also extreme where the different amounts of superphosphate (fig. 3) were used. These results were interpreted as supporting the field observations and showing quite definitely that the ability of the lime to reduce chlorosis was due to the magnesium it contained, and not to any effect on aluminum toxicity. If the chlorosis were due to aluminum toxicity, the superphosphate and chemically pure lime treatments should have reduced the chlorosis just as much as did the agricultural lime. It would also be necessary to consider magnesium an antidote for aluminum poisoning, but for this there is no experimental support.

To check further the aluminum hypothesis, lettuce, an aluminum-sensitive crop, was grown in pots of two kinds of soil. The first of these soils produced extremely chlorotic corn in the field while the second produced corn practically free of chlorosis. In pots the corn behaved exactly as it did in the fields. The growth of lettuce on these two soils was similar and showed no evidence of aluminum injury. The average dry yield of lettuce per pot of three plants for the first soil was 8.158 gm. and for the second 7.509 gm., giving a difference of 0.649 gm. In view of the variations among the pots this difference was considered insignificant.

Another point bearing on the aluminum hypothesis was the fact that the soil on which the extremely chlorotic corn was produced has a pH value of 5.4. In view of the work of Burgess and Pember, such a soil reaction would hardly be expected to be associated with sufficient

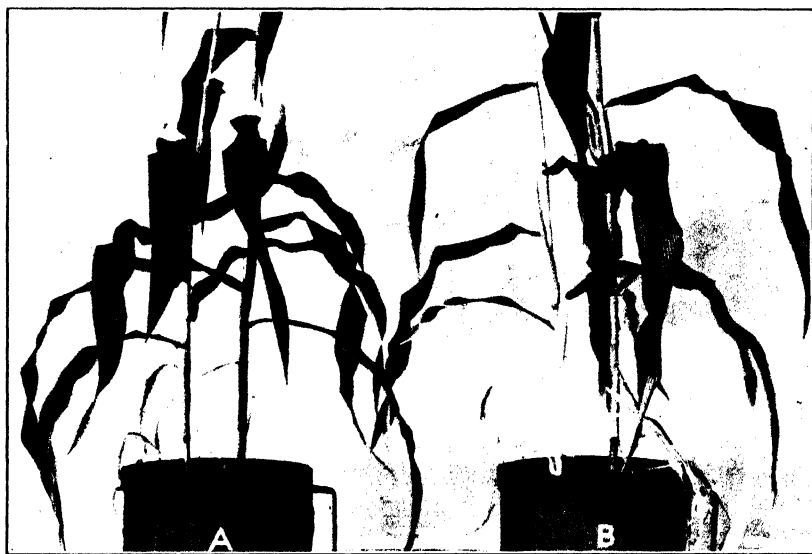


FIGURE 4.—The comparative effect of magnesium sulphate and chemically pure lime as preventives of chlorosis in corn grown on soil from fields which produced chlorotic corn: A, Magnesium sulphate was applied to the soil in this pot at the rate of 400 pounds per acre; B, chemically pure calcium carbonate was applied at the rate of 2 tons per acre

active aluminum to affect a plant so resistant as corn. The limed area of the field had a pH value of 6.1.

CHEMICAL STUDIES

When the chlorosis studies were first undertaken an attempt was made to obtain evidence on the aluminum hypothesis by chemical analysis of the plant. From the 1924 crop samples of corn showing slight, medial, and extreme chlorosis were taken. Iron and aluminum determinations were made on the roots and stalks. Hoffer (6) has called attention to the fact that when aluminum is available in the soil it will accumulate in the stalks of corn, particularly in the vascular plate tissue of the node. His results also indicated a relation between the aluminum content of stalks and injury due to corn root rots.

It was because of this work that the first chemical studies were confined to the stalk and roots. The removal of soil from the roots was not so easily accomplished as might have been desired. Although every precaution was taken to clean the roots, when the sample was ground there was evidence that a slight amount of soil was included.

In Table 10 are presented the results of the chemical analyses. The iron determinations on the stalks show no significant differences between the normal and the chlorotic plants. There appears, however, to be more iron in the roots than in the stalks, but this difference might possibly be accounted for by the small amount of soil unavoidably included in the sample. However, if this explanation is correct there should also be more aluminum in the roots than in the stalks, and such is not consistently the case.

The aluminum determinations showed higher amounts in the normal than in the chlorotic plants for both the stalks and the roots. This may be taken to indicate that accumulation of aluminum in the chlorotic plants was not responsible for the chlorosis. In addition, there was no evidence of any root rot associated with the chlorosis. According to Hoffer (6), the absence of root rot may be an indication that aluminum was not a factor.

TABLE 10.—*Influence of chlorosis on aluminum and iron content of corn stalk and roots*

Part of plants	Description of plants	Content of—	
		Fe ₂ O ₃	Al ₂ O ₃
		<i>Per cent</i>	<i>Per cent</i>
Stalks	Normal	0.013	0.193
	Slightly chlorotic	.010	.110
	Medially chlorotic	.013	.019
	Extremely chlorotic	.014	.029
Roots	Normal	.034	.171
	Slightly chlorotic	.029	.091
	Medially chlorotic	.022	.115
	Extremely chlorotic	.032	.136

In 1927 samples were taken again of normal and extremely chlorotic plants. The normal plants were taken from plot 1A, a plot practically free of chlorosis; the chlorotic plants from plot 2D, where chlorosis was most severe. An ash analysis was made.³ In preparing the samples for analysis it was decided to exclude the roots because of the difficulty in getting a satisfactory sample and because it was evident that an analysis of the top part of the plant would be sufficient. The samples in each case were separated into nodes, internodes, leaf sheath, and leaf blade. This separation was made just after the plants had been dried.

The results of the analytical work are given in Table 11. In accord with the results obtained on the 1924 crop, no consistent differences in iron and aluminum content of the normal and chlorotic plants were apparent. In the nodal tissue, where the aluminum would be expected to accumulate, 0.06 per cent was found in the normal and 0.05 per cent in the chlorotic plants. Taking the plant as a whole.

³ The writer is indebted to Dr. G. N. Hoffer, of the Indiana Agricultural Experiment Station, for his interest in this problem and for his assistance in having these analyses made in his laboratory.

the iron and aluminum content of the normal plants was very similar to that of the chlorotic plants.

The ash content of the leaf sheath and leaf blade was higher in the normal than in the chlorotic plants, while the reverse was true of the nodes and internodes. The average for the whole plant showed very little difference between the normal and chlorotic plants. The leaf sheath and leaf blade contained significantly more ash than the nodes and internodes.

TABLE 11.—*The effect of chlorosis on the mineral constituents of different parts of the corn plant*

Mineral	Percentage content of—								Average	
	Nodes		Internodes		Leaf sheath		Leaf blade		Normal	Chlorotic
	Normal	Chlorotic	Normal	Chlorotic	Normal	Chlorotic	Normal	Chlorotic		
Total ash	5.56	8.70	3.85	6.72	9.11	8.58	12.29	8.99	7.70	8.25
SiO ₂	.46	.45	.78	.67	3.72	2.94	5.88	3.51	2.71	1.89
P ₂ O ₅	.26	.20	.20	.16	.45	.40	.86	.81	.44	.39
Al ₂ O ₃	.06	.05	.04	.06	.08	.11	.09	.06	.07	.07
Fe ₂ O ₃	.01	.01	.01	.09	.07	.07	.07	.06	.04	.06
CaO	.99	.66	.47	.38	1.03	.74	1.67	.88	1.04	.66
MgO	.34	.10	.16	.06	.40	.12	.33	.11	.31	.10
K ₂ O	2.32	4.79	1.76	3.82	2.72	3.33	2.80	2.86	2.40	3.70

Silicon, phosphorus, and calcium were all found to be slightly more abundant in the normal than in the chlorotic plants. These elements were more plentiful in the leaf sheath and leaf blade than in the other parts of the plant. Plot 1A, where the sample of normal plants was taken, had received more phosphorus and calcium than plot 2D, where the sample of chlorotic plants was taken. This might explain the higher percentages of these two elements in the normal corn. Plot 2D had received more potash than plot 1A, and Table 11 shows consistently more potash in the chlorotic plants.

While the differences just mentioned were quite consistent they were not so large as those for magnesium. Table 11 shows on an average more than 300 per cent more magnesium oxide in the normal than in the chlorotic plants. This appeared to be the most significant result of the chemical analyses.

The field work showed that both lime and magnesium sulphate counteracted chlorosis; the greenhouse work showed that it was the magnesium in the lime which prevented chlorosis; and the chemical work now shows that the important chemical difference between the normal and chlorotic plants is in the magnesium.

DISCUSSION

The data presented show that the chlorosis on North Corn Acre was due to a lack of magnesium. The conditions which brought about the shortage of magnesium were: (1) The removal of magnesium from the soil by leaching and continuous cropping, and (2) the use of fertilizers practically free of magnesium for a long period.

Crops in general do not remove large quantities of magnesium from the soil. It has been estimated from the work of Lyon and Bizzell (9)

that corn may take between 15 and 20 pounds per acre. Jenkins (7) has estimated that a crop of tobacco removes in its leaves and stalks about 15 pounds per acre. According to these estimates the magnesium requirements of corn and tobacco are similar.

More important than the removal of magnesium by crops is the loss by leaching. According to the lysimeter experiments of Lyon and Bizzell (9), the magnesium lost by drainage may vary from 28 to 70 pounds per acre. On North Corn Acre the soil was much lighter than that with which Lyon and Bizzell obtained these results, indicating that even greater leaching may have taken place than was observed by these writers.

The belief that leaching has been a factor in lowering the level of magnesium on North Corn Acre is supported by the fact that chlorosis increased with increased rainfall. Garner and his associates (4) emphasized the fact that plants on light soils suffer most from sand drown. They also stated that excessive rainfall causes sand drown to become more widespread.

In view of the fact that the fertilizers used for a long period on North Corn Acre were practically free of magnesium, it was to be expected that the losses by drainage and crop removal should in time exhaust the soil. Because of its magnesium content, applications of farm manure might have prolonged the time before the appearance of chlorosis, but it is doubtful whether the manure, unless applied in very large amounts, would have maintained the magnesium supply indefinitely.

It has not been necessary up to the present for workers in the field of soil fertility to pay much attention to magnesium. They have been concerned chiefly with the commonly deficient nutrients, nitrogen, phosphorus, and potash. With continuous cropping, however, and the use of fertilizers practically free of magnesia, particularly on soils subject to considerable leaching, magnesium deficiency is likely to become more and more a problem.

As manure becomes less available, successful crop production must depend upon fertilizer chemicals. The introduction of higher analysis fertilizers means materials of greater purity. Thus the fertilizer practice of the future, unless attention is given to magnesium, is likely to assist in depleting the soil magnesium. In the fertilizer practice of the past magnesium has been contained in many of the low-grade materials as an impurity. However, it should be borne in mind that the soil conditions responsible for the magnesium deficiency here reported have developed with the use of the medium-analysis fertilizer materials commonly employed for the past 30 years.

Materials which may be used as sources of magnesium are lime, sulphate of potash-magnesia, vegetable organics, magnesium sulphate, and basic slag. The proper amount of these materials to use will depend on the amount of magnesium they carry and on how severely the crop may be suffering from lack of it. Garner and his associates (4) have estimated that about 20 pounds per acre of magnesium is sufficient to control sand drown. In the light of evidence secured by the writer this quantity may need modification to meet the variation in requirements induced by differences in rainfall and soil conditions. For example, during the season of 1928 sand drown was noted on tobacco on certain plots where approximately 30 pounds of magnesium

per acre were applied in the form of sulphate of potash-magnesia and cottonseed meal. The soil on which this observation was made was light and was situated on a slope, thus being subject to both leaching and erosion during a rainy season. For corn the experience on North Corn Acre indicated that between 20 and 40 pounds of magnesium per acre are sufficient to control chlorosis. Probably for average conditions applications of between 20 and 25 pounds per acre will give satisfactory results. In view of the amounts removed by drainage as reported by Lyon and Bizzell (9), it would hardly seem that the estimates above are adequate. Since the results in the field show them to be sufficient the indication is that some magnesium must be released by the soil.

SUMMARY

The work presented in this paper consisted in a study to determine the cause of chlorosis on corn growing in one of the experimental fields at the Massachusetts Agricultural Experiment Station. Two hypotheses were put forward to explain the chlorosis (1) aluminum toxicity and (2) lack of magnesia in the soil. Preliminary observations indicated that lime possessed the ability to control the chlorosis. Magnesium sulphate treatments were made for comparison with the lime treatments, and it was found that both treatments counteracted the chlorosis in the field. In the greenhouse, however, where magnesium sulphate was compared with chemically pure calcium carbonate, only the former was capable of controlling chlorosis. Antidotes for aluminum toxicity such as high applications of superphosphate had no influence on the chlorosis. Chemical analyses always revealed a larger amount of magnesium in the normally green than in the chlorotic plants. Aluminum, on the contrary, proved to be as abundant in the normal as in the chlorotic plants. Thus the conclusion was reached that lack of magnesia was responsible for the chlorosis.

Chlorosis of corn and sand drown of tobacco both became more injurious when heavy rainfalls occurred during the growing season. The yield of corn was found to be more affected by chlorosis than that of the stover, the latter being reduced only slightly.

The conditions that favor magnesia hunger are a light leachy soil, the use of chemical fertilizers free of magnesium and without manure, and continuous cropping. If the leachings from lysimeters are taken as a criterion, the removal of magnesia by drainage water is of greater consequence than its removal by crops.

In fertility programs of the future it may be necessary to consider magnesium more than in the past, especially for some of the lighter soil types. This seems particularly important in view of the fact that fertilizer mixtures are likely to be composed of purer chemicals.

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CAN NODULE BACTERIA OF LEGUMINOUS PLANTS FIX ATMOSPHERIC NITROGEN IN THE ABSENCE OF THE HOST? ¹

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INTRODUCTION

Nitrogen-fixation studies with nodule bacteria have been reported at frequent intervals during the period since 1886-1888, when Hellriegel and Wilfarth announced that these bacteria can live in symbiosis with leguminous plants and make the growth of the latter largely independent of the nitrogen content of the soil. The findings of these two men have been verified repeatedly and are to-day practically universally accepted by research workers in this field. We know that the nodule bacteria are necessary for nitrogen fixation to take place in the leguminous plants, but we do not know the part played by these bacteria in the fixation process. The prevailing theory that the bacteria obtain carbohydrates from their host, use the energy for growth and nitrogen fixation, and in turn supply the host with nitrogen, is far from being proved. If the bacteria fix as large quantities of nitrogen as the above theory implies, then it should presumably be easy to demonstrate the fixation by the bacteria growing independent of the host. Numerous attempts have been made to do this with widely varying results. A résumé of some of the outstanding experiments along this line is given below. These are arranged for the most part in chronological order.

HISTORICAL REVIEW

Beijerinck (3) ³ obtained fixations varying from 0.9 to 1.8 mgm. N per 100 c. c. of media in a medium containing 5 to 7 mgm. of nitrogen. He considered that the data proved that the organism could fix nitrogen but that the increases were not greatly in excess of the errors of analysis.

Prazmowski (32) stated briefly, without giving data, that he had shown that the legume organism can fix nitrogen when grown on a nitrogen-free medium, but the power of fixation was not great.

Frank (10), in a review of his own investigations, concluded that it was very questionable whether *Rhizobium* had the power of fixing free nitrogen independent of the host, and was of the opinion that more likely it acted merely as a stimulus to the plant, rendering the inherent ability of the latter for nitrogen assimilation more active.

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² The writer wishes to thank E. B. Fred for supplying a portion of the cultures of *Rhizobium* used in these investigations, and for suggestions given; also F. G. Cottrell and others of the laboratory staff for their interest and help during the progress of the work.

³ Reference is made by number (italic) to "Literature cited," p. 922.

Immendorff (22) was unable to secure appreciable growth or nitrogen fixation by legume bacteria growing on various nitrogen-free media. In order to secure good growths combined nitrogen was necessary in the medium.

Berthelot (4) grew lupine bacteria on a humic-acid medium and obtained an increase of 5.3 mgm. N in four months. For inoculation the juice from the roots of an inoculated lupine was used. Apparently a pure culture was not used, and hence the results are of little significance from the standpoint of the question under consideration.

Gonnermann (16) stated that the root-tubercle bacteria alone could not make the free nitrogen available for the plant, but considered that the plant itself without symbiosis could use free nitrogen.

Stutzer, Burri, and Maul (38) grew bacteria in 2-liter flasks, containing 50 c. c. of nitrogen-free medium absorbed in 10 gm. asbestos. After two months the analyses showed gains of 3.25, 2.23, and -0.48 mgm. N for three experiments. The authors did not consider that the results justified a definite statement as to the nitrogen-fixing power of *Rhizobia*.

Mazé (26, 27) claimed to have demonstrated that legume bacteria can fix nitrogen if grown on a suitable organic medium, such as legume extract containing the necessary mineral elements and sugar. He was not able to secure appreciable fixation on a nitrogen-free medium. This work has been quoted so many times as proof that the legume bacteria can fix nitrogen that a critical study of the articles is very desirable. In the first place, the statements regarding nitrogen fixation are based on only about 25 or 30 analyzed cultures. The purity of these cultures was not adequately tested, and from his statements we can almost say with certainty that they were mixed. He observed a strong odor of cheese given off by the cultures and in some cases a rapid liquefaction of gelatin. These properties are certainly not characteristic of *Rhizobia*. He found that the bacteria decomposed sucrose very rapidly with the production of CO_2 . Within 15 days usually 2 per cent sugar solutions were almost wholly converted into CO_2 with a corresponding oxygen consumption. In the first of Mazé's two articles referred to here, he reports three experiments, showing fixations of 40.8, 47.5, and 23.4 mgm. N per 100 c. c. in 15 or 16 days, starting with a medium already rich in nitrogen. In the second article the fixations in three additional experiments were 12.1, 12.8, and 15 mgm. N per 100 c. c. In the third article the fixations were -3.7, -2.4, 2.2, 0.8, 2.2, and 2.4 mgm. N in one series and 3.3, 6.0, 0.4, 2.9, and 6.3 mgm. N in another series. Most of these results were secured on media which already contained more nitrogen than the organisms would ordinarily need. The work is interesting from the historical standpoint, but in the light of our present knowledge of legume bacteria we can not seriously consider it as proof of the fact that *Rhizobia* can fix nitrogen independently of the host.

Greig-Smith (17) stated:

Experiments were made with pure cultures of the organism, using glucose and sucrose in conjunction with plant extract, but neither with *Rhizobia* obtained from the lupin nor the pea could any fixation of free nitrogen be found either in faintly acid, neutral, or faintly alkaline media; the cultures finally contained the same amount of nitrogen as they had at the beginning of the experiment.

He reported also that he was unable to obtain any nitrogen fixation with nodule bacteria, grown on various media. Later (18) he reported numerous analyses showing some nitrogen fixation in artificial cul-

tures. In this later work he concluded that the fixation was, in general, "coincident with and proportional to the formation of slime. Under conditions that preclude the formation of slime, there is no fixation."

Neumann (30) obtained fixations varying from 4.4 to 49.9 mgm. N in soil and legume extracts inoculated with mixed cultures from soil, roots, and nodules, and stems and leaves. These results are of little significance from the standpoint of this discussion since pure cultures of Rhizobia were not used.

Löhnis (25) secured nitrogen fixation on a soil-extract medium, the quantities varying from 0 to 1.54 mgm. N per 100 c. c. of medium in 21 days.

Moore (28) obtained fixations of 0.2 to 2.2 mgm. N where various strains of legume bacteria were grown in an aerated nitrogen-free medium. The nitrogen was found to be stored up within the bacterial cells as protein and could not be removed by merely filtering off the medium.

Lewis and Nicholson (24) report fixations with alfalfa bacteria varying from 0 to 16.2 mgm. N per 100 c. c. of media, with an average of approximately 2.2 mgm. N. The results appear to be rather erratic, the largest fixations being secured in media which contained far more nitrogen than the bacteria would be expected to assimilate under the conditions.

The work of Golding (15) has probably been more widely quoted in recent years as proof that nodule bacteria can fix nitrogen than that of any other worker. It has been repeatedly stated in the literature that he demonstrated that by the continued removal of the soluble products of growth from bacterial cultures they fixed comparatively large quantities of nitrogen. Let us see what Golding, himself, said. In his first experiment a gain of 342 mgm. N was obtained where 520 gm. of macerated legume stems, leaves, and nodules were present in 3,000 c. c. of medium, which was constantly being removed by suction. This medium was not sterilized and hence the results mean nothing so far as demonstrating that legume bacteria can fix nitrogen. In a second experiment 500 c. c. of fresh unheated cold-water extract of pea plants with the addition of 5 gm. of dextrose served as a medium. In this impure culture the fixation was 24.4 mgm. N, again showing nothing regarding the ability of root-nodule bacteria to fix nitrogen, because pure cultures were not used. In a third experiment, using a synthetic nutrient medium containing 1,550 c. c. of sterile pea extract inoculated with a pure culture, a fixation of 31.5 mgm. N was obtained in 13 days. In a similar pure-culture experiment, starting with 250 c. c. of sterile plant-extract medium, the fixation was 7 mgm. in 22 days. The quantity of nitrogen fixed was, therefore, 2.0 and 2.8 mgm. N per 100 c. c. of medium, respectively, for the two pure-culture experiments. This fixation is no greater than that reported by certain other investigators as occurring in ordinary culture flasks and is not greatly in excess of experimental error, considering the rather difficult technic used and the fact that plant extracts were employed. It is very difficult to secure accurate analyses with such material, and even if closely agreeing duplicate determinations are obtained there is no guarantee that the analyses are really accounting for every form of nitrogen present. The recent paper of Christensen (9) is of interest in this connection. It is also worth while to empha-

size that Golding like Mazé obtained his fixations with *Rhizobium* grown on a medium already containing more nitrogen than the bacteria needed. Certainly, numerous repetitions of the work would be necessary to make the results really conclusive. Golding tried various other methods to demonstrate fixation in pure cultures, but without success. Results were unsatisfactory where agar cultures were floated on sugar solutions in sterile parchment dialyzers; likewise, where cultures were grown in flasks in thin layers; and again where sugar was added to the cultures daily.

Fred (11), using 15 strains of legume bacteria, reported fixations varying from 17 to 34 mgm. N per 200 c. c. of a nitrogen-deficient medium. However, in a private communication he states that there was an error in printing and that the data should be based on 1,000 c. c. instead of 200 c. c. of media. The average fixation was, therefore, about 1.5 mgm. N per 1,000 c. c. In a second paper (12) he reported fixation for several strains of nodule bacteria grown in liquid, sand, and soil cultures. The approximate average nitrogen gain for the pure cultures was 8.9 mgm. N per 1,000 c. c. of liquid media, 8.5 mgm. N per 100 gm. sand, and 5.2 mgm. N per 100 gm. clay soil. The author summarized the work as follows:

While the nitrogen assimilation in the liquid medium, in the sand, and in the soil, was very small, it was sufficient to give a reasonable basis for the belief that *Bacillus radicolica* will live in the soil without the host plant and accomplish a certain amount of nitrogen assimilation.

In a later report (13) of a rather extensive series of experiments, he obtained fixations of usually less than 1 mgm. N per 100 c. c. with a few increases as high as 3 mgm. N. The results were usually higher on agar cultures than in solution. The presence of a trace of nitrogen seemed to aid in the gain, while large amounts retarded the process. He stated that the bacteria fixed as much, or more, nitrogen per unit of carbohydrate consumed than such a form as *Azotobacter*. In a bulletin by Fred, Whiting, and Hastings (14) fixations of 14.15 and 18.55 mgm. N were obtained per 15,000 c. c. of media in two experiments where legume bacteria were grown on Berkefeld filters with provision for the constant addition of fresh medium and removal of the old. Most of the nitrogen assimilated was held in the residue on the candle and only a very small part was carried into the filtrate.

Rossi (34) made a study of the nitrogen-fixing power of legume-nodule bacteria grown on gelatin, agar, and liquid media and obtained negative results. An experiment conducted somewhat along the lines of Golding's (15) work also showed no significant gain in nitrogen.

Buchanan (8) reported the results of a detailed study of the gum produced by *Bacillus radicolica* and stated that it contained no combined nitrogen.

Bottomley (5) secured a fixation of 2.3 mgm. N per 100 c. c. of synthetic nitrogen-free medium with nodule bacteria. He repeated the work later (6), making the analyses after all sugar was used up, and secured similar results. In 1912 (7) he isolated the bacteria from the root nodules of *Myrica gale* and found them identical in structure and growth with the legume nodule bacteria. In liquid cultures a fixation of 2.05 mgm. N per 100 c. c. was obtained in seven days.

Spratt (37) also isolated bacteria from the nodules of nonlegumes, Podocarpaceae, found them identical with *Pseudomonas radicolica* obtained from the Leguminosae, and secured a gain of 2.5 mgm. N

by the organism grown in pure culture. In another paper (36) she reports fixations of 2.5 to 3.5 mgm. N by *P. radicola* isolated from the root nodules of *Alnus* and *Elaeagnus* and grown in pure culture in laboratory media.

Herke (20) determined the nitrogen-fixing power of legume bacteria and concluded that a soil extract medium favors fixation, the rate being an average of about 0.4 mgm. N per 100 c. c. of medium in unaerated cultures. Increasing aeration, either by the use of thin layers of media or by bubbling air through the cultures, slightly increased fixation. Where the action of the plant was imitated by growing the organisms in funnels on kieselguhr with the periodic addition of mannit solution and removal of the old medium by suction, the fixation varied from 1.19 to 3.08 mgm. N per flask.

Olaru (31) reported fixations varying from 1.5 to 32.1 mgm. N per 100 c. c. of media in plant extracts containing 2 per cent sucrose and varying percentages of MnSO_4 , inoculated with pea-nodule bacteria.

Mulvania (29) found that where a dialyzing membrane intervened between a legume bacterial culture and the roots of the host plant no infection of the roots occurred and the plants did not benefit by the presence of the bacteria, showing that no usable dialyzable nitrogen was produced by the bacteria.

Rocaolano (33), starting with a medium containing 28.9 mgm. N per 100 c. c., added MnCl_2 in amounts varying from 0.001 to 0.02 gm. of Mn ion per 100 c. c. of medium. The nitrogen fixation by the nodule bacteria increased from 3.1 mgm. in the control to 9.6 mgm. with 0.006 gm. of Mn ion per 100 c. c. With higher concentrations the fixation decreased markedly.

Hills (21) claimed to have demonstrated that *Rhizobia* fixed a small quantity of nitrogen, varying from 0.15 to 0.43 mgm. N per 100 c. c. of media, when grown on agar films. He claimed, further, that the addition of nitrates increased the fixation slightly, the increases under these conditions varying from about 0.53 mgm. to 3.5 mgm. N per 100 c. c. A critical study of the data shows, however, that the method of analysis used accounted for less than half of the nitrogen actually added to the cultures as nitrate.

Joshi (23) inoculated three strains of nodule bacteria separately into soil-extract-mannit media and determined the nitrogen fixation after 10 days. The increase in nitrogen, due to the bacteria, varied from 0.8 to 1.2 mgm. N per 100 c. c. Several additional strains gave an average fixation per 100 c. c. of 1.4 mgm. N with a maximum of 2 mgm. N. No details of the analytical methods are given and only a few figures.

Barthel (2) attempted to establish definitely whether leguminous nodule bacteria possess the faculty of fixing nitrogen, when grown in pure cultures, and obtained negative results with a culture isolated from the pea.

Halversen (19, p. 400) reported fixations of 1.2 and 1.7 mgm. N by *Rhizobium leguminosarum* grown for eight days on synthetic media. He states that the amounts of nitrogen fixed "are so small for the *Rhizobium* cultures that the experimental error is often greater than the amount of the determination." In one case, however, he obtained a fixation of 7.2 mgm. N in a culture containing lime, which was aerated for four weeks.

Skinner (35) obtained no fixation with alfalfa bacteria and reports that the finding was entirely unexpected.

The writer (1) in a preliminary statement reported that red-clover bacteria failed to fix nitrogen when grown on plant-extract media.

A study of the investigations discussed above emphasizes certain outstanding points: (1) Rarely has *Rhizobium* been reported as making a good growth or showing fixation appreciably above the experimental error when grown on a nitrogen-free medium. (2) Where small amounts of nitrogen were present, particularly in miscellaneous unknown forms such as soil extract, gains of nitrogen have been frequently reported but these increases have usually been less than 1 mgm. It should also be borne in mind that under the same conditions in a number of instances no increases of nitrogen have been secured and a number of losses have been recorded. (3) When large quantities of nitrogen were present in the medium at the start the results have been very much more erratic, showing in some cases marked losses and in others increases up to about 50 mgm. N. Fairly large fixations of nitrogen have frequently been reported for legume-nodule bacteria grown in plant-extract media. Usually where the largest increases were obtained the bacteria were grown in media that contained from two to five times as much nitrogen as the bacteria would need for a normal growth, assuming no fixation. It is true that not all of the nitrogen in media, such as plant extracts, is available, but certainly a good percentage is. A further important fact brought out by a careful study of the references given is that the conclusions in most instances have been based upon a very limited amount of data and very few extensive studies of the question have been reported. Much of the earlier work is questionable because very obviously pure cultures were not always used.

EXPERIMENTAL WORK

In an attempt to clarify the situation a rather extensive series of experiments was initiated at this laboratory in May, 1923, and these have been continued to the date of writing, December, 1928. Most of the work reported in this paper was completed more than two years ago but not published because of the desire to test thoroughly the numerous suggestions that have been given in the literature and by personal contacts. Because of the fact that the results reported below are of the negative type a logical presentation is impossible. The experimental work consisted mostly in testing the nodule bacteria for nitrogen fixation under as many different conditions as possible. Failing in one experiment to obtain nitrogen fixation, other experiments were started which frequently had little bearing on those previously conducted, except that they may have been designed to bring some additional factor or factors into the picture. These heterogeneous experiments are, therefore, grouped together and presented below.

METHODS

Many schemes were tested by the writer for the growth of *Rhizobia* in special types of apparatus designed to favor fixation, but in most instances the organisms were grown at 28° C. in 100 c. c. portions of

media contained in 300 c. c. Erlenmeyer flasks, and unless otherwise stated, it may be assumed that this was the procedure used in all experiments reported here. In many cases flasks having capacities up to 3 liters were used in order to provide greater aeration. The composition of the medium was the chief variable. As a basis for most of the media, Ashby's solution with slight modifications was chosen. When the term "Ashby's solution" is used in the following pages, a solution of the following composition is meant:

	Grams		Grams
Magnesium sulphate.....	0.2	Ferric chloride.....	0.0005
Dipotassium phosphate.....	.2	Calcium carbonate.....	1.0
Sodium chloride.....	.2	Water.....	1,000.0
Calcium sulphate.....	.1		

The sugar used was usually 1 per cent dextrose, but this varied and reference must be made to each individual experiment. To this medium as a basis various additions were made and their influence, if any, on nitrogen fixation determined.

Nitrogen determinations, unless otherwise stated, were made by the Kjeldahl method, using CuSO_4 , K_2SO_4 , and H_2SO_4 for digestion. In some of the experiments, reported near the end of this paper, analyses were made by the Kjeldahl method, using metallic mercury and H_2SO_4 for digestion, followed by precipitation of the mercury with sodium thiosulphate prior to distillation.

NITROGEN-FIXATION STUDIES ON NITROGEN-FREE MEDIA

The usual method of testing a microorganism for nitrogen-fixing power is to grow it in pure culture in a liquid, or in some cases solid, medium containing the necessary inorganic elements for its growth and a suitable carbohydrate. Ordinarily organisms show their highest rates of fixation on such a nitrogen-free medium or at least on a medium containing only a comparatively small quantity of nitrogen. As the supply of available nitrogen is increased appreciably fixation decreases. In other words the microorganism uses atmospheric nitrogen only when it is forced to do so and prefers the simple nitrogenous salts.

The first experiment started, and much of the later work, was based on this idea that in order to demonstrate nitrogen fixation the legume bacteria should be grown under starvation conditions, so far as fixed nitrogen is concerned. In this first experiment two media were chosen, Ashby's solution with the addition of 1 per cent mannitol and Hunter's medium, consisting of magnesium sulphate 0.2 gm., dipotassium phosphate 0.5 gm., sodium chloride 0.2 gm., and mannitol 10 gm. per liter, adjusted to pH 7.0 to 7.4. Cultures were grown both with and without CaCO_3 . A portion of the flasks were inoculated with soybean bacteria and others with a culture of *Azotobacter vinelandii* obtained from the New Jersey Agricultural Experiment Station. Optimum growth conditions for the two organisms are similar and hence the use of *Azotobacter* served as a check on the soybean culture. Table 1 gives the analyses after 16 and 24 day growth periods at room temperatures.

TABLE 1.—Results of nitrogen-fixation tests with soybean bacteria and *Azotobacter vinelandii* grown on two different media and incubated at room temperature

ASHBY'S MEDIUM									
Culture	Incubation period	With calcium carbonate				Without calcium carbonate			
		Growth observations	N	Average	N fixed	Growth observations	N	Average	N fixed
	Days		Mgm.	Mgm.	Mgm.		Mgm.	Mgm.	Mgm.
Control, uninoculated.	16		0.0	0					
Soybean bacteria	16	Slight	0.04	0.02	0.02	Slight	-0.03	-0.02	-0.02
<i>A. vinelandii</i>	16	Good	6.23	6.23	6.23	Good	6.62	5.98	5.98
Soybean bacteria	24	Slight	-0.11	-0.04	-0.04	Slight	5.34	-0.11	-0.11
<i>A. vinelandii</i>	24	Good	5.17	4.98	4.98	Good	5.36	5.50	5.50

HUNTER'S MEDIUM									
Culture	Incubation period	Growth observations	N		N fixed		Growth observations	N	
			0.14	Lost.	0.14	0.14		0.11	0.06
Soybean bacteria	16	Slight	5.45	5.84	5.65	5.65	Slight	3.11	0.06
<i>A. vinelandii</i>	16	Good	-0.01	-0.04	-0.03	-0.03	Fair	3.70	3.41
Soybean bacteria	24	Slight	5.11	4.85	4.98	4.98	Slight	-0.13	0.06
<i>A. vinelandii</i>	24	Good	3.19	3.45	3.32	3.32	Fair	3.19	3.32

It will be observed that the soybean organisms did not fix nitrogen in either of the two media used whether in the presence or absence of calcium carbonate. The results in all cases are wholly within the experimental error. So far as growth of the soybean bacteria was concerned the observations checked with the analysis, about the only evidence of growth being a slight amount of gum formation.

The culture of *Azotobacter vinelandii* fixed nitrogen under all conditions, the quantity being greatest in Ashby's medium containing calcium carbonate and lowest in Hunter's medium without calcium carbonate. It will be observed, further, that in every instance the quantity of nitrogen found in the flasks inoculated with *Azotobacter* was greater at the end of 16 than of 24 days. This was probably due to the loss of a volatile form of nitrogen from such cultures.

NITROGEN-FIXATION STUDIES WITH VARIOUS STRAINS OF NODULE BACTERIA

Another experiment, similar to that reported in Table 1, was started soon after its completion for the purpose of testing cultures from a large number of species of legumes. The experimental methods were, in general, the same except that the temperature of incubation was 25° C. and a soil-extract medium, prepared according to the recommendations of Löhnis, was substituted for Hunter's medium. This contained in addition to the extract, 0.5 gm. dipotassium phosphate, 10 gm. mannitol, and excess of calcium carbonate in a liter of water. The Ashby's solution used contained 20 gm. of mannitol per liter. The bacterial cultures used had been tested for purity and nodule production. Because of the large number of cultures tested and the desirability of using two media, duplicate determinations were not made. The analyses for total nitrogen were made at the

end of 32 days. Prior to making the final analyses nitrate determinations were made on the uninoculated media and 0.2 mgm. nitrate nitrogen found per 100 c. c. Qualitative tests made on several of the cultures showed no nitrate present, as was to be expected, and hence the reduction method to include nitrates in the total nitrogen determinations was used only in the analysis of the control flasks. The data are given in Table 2.

TABLE 2.—Results of nitrogen determinations made on various *Rhizobium* cultures grown on two different media and incubated at 25° C. for 32 days

Culture	Ashby's medium			Löhnis' soil-extract medium		
	Growth observations	N	N fixed	Growth observations	N	N fixed
		Mgm.	Mgm.		Mgm.	Mgm.
Arlington soybean	Slight	0.02	0.02	Fair	2.00	0.17
Virginia soybean	do	.06	.06	Slight	1.81	— .02
Tokyo soybean	do	.03	.03	Good	1.70	— .13
Kidney bean	do	.02	.02	do	1.83	0
Japan clover	do	.07	.07	Slight	1.81	— .02
Vetch	do	.03	.03	Good	1.85	.02
Crimson clover	do	.06	.06	do	2.14	.31
Trifolium agarium	do	.04	.04	do	1.71	— .12
Velvetbean	do	0	0	Slight	1.76	— .07
Admiral garden pea	do	.03	.03	Good	1.77	— .06
Lima bean	do	.03	.03	Slight	2.11	.28
Alfalfa	do	.01	.01	do	2.14	.31
Petit pois garden pea	do	0	0	do	1.81	— .02
Red clover	do	.01	.01	Good	1.83	0
Sweetclover	do	.01	.01	Slight	1.85	.02
Lotus corniculatus	do	.02	.02	do	1.85	.02
Sulla	do	.01	.01	do	1.70	— .13
Cowpea	do	.01	.01	do	2.07	.24
Garden bean	do	0	0	Fair	1.78	— .05
New Era cowpea	do	— .01	— .01	Slight	1.83	0
Check, uninoculated	do	0	—	do	1.88	—
Do	do	0	—	do	1.83	—
Do	do	—	—	do	1.79	—

The results seem to show, beyond any reasonable degree of doubt, that under the conditions of the experiment no nitrogen was fixed by the cultures. In the case of Ashby's medium the analyses agree unusually closely and all variations from the control are easily within experimental error. The analyses of the soil-extract medium show slightly wider variations, there being a few increases over the check of 0.2 to 0.3 mgm. N and also a few losses. The average of all the results gives a figure slightly on the positive side, but the increase is not significant. Observations of growth of the various cultures showed no correlation with the nitrogen determinations; for instance, most of those which gave 0.2 to 0.3 mgm. N increase over the control were usually the ones which showed almost negligible growth.

In addition to the results reported here, six other cultures on Mazé's bean-extract medium were run at the same time. The analyses of these showed slight losses in all cases.

NITROGEN-FIXATION STUDIES ON "RADICICOLA" SOLUTION

A medium which has been used frequently for the growing of legume bacteria is "Radicicola" solution, mentioned by Lipman and Brown in their Laboratory Guide for Soil Bacteriology. It consists of 10 gm. of saccharose and 1 gm. of dipotassium phosphate, dissolved in 1 liter

of tap water. In order to expose a large surface of the medium to the air, 100 c. c. portions were placed in 2-liter Erlenmeyer flasks. The flasks were inoculated with six strains of *Rhizobia* and allowed to incubate for 97 days at room temperature. The period was made long because some of the investigators previously mentioned reported greater fixations over long intervals. Good gum formation was obtained for the two red-clover cultures and with the soybean and cowpea bacteria. The total nitrogen determinations are given in Table 3.

TABLE 3.—Results of nitrogen determinations on various *Rhizobium* cultures grown on Radicicola solution and incubated at room temperature for 97 days

Culture	Growth observations	N	Average N	N fixed
		Mgm.	Mgm.	Mgm.
Red clover (1).....	Fair.....	0.39	0.44	—0.07
Red clover.....	do.....	.49		
Red clover (2).....	do.....	.62	.61	.10
Red clover.....	do.....	.59		
Winter pea.....	Slight.....	.39	.44	—0.07
Do.....	do.....	.49		
Sweetclover.....	do.....	.60	.53	.02
Do.....	do.....	.46		
Soybean.....	Fair.....	.39	.49	—0.02
Do.....	do.....	.58		
Cowpea.....	do.....	.56	.56	.05
Do.....	do.....	.56		
Control, uninoculated.....		.46	.51	
Do.....		.56		

The evidence again shows very definitely that no nitrogen was fixed with any of the cultures, the variations in all cases being within experimental error. This was to a certain extent unexpected prior to analysis because the growth in the flasks seemed unusually good in some cases. The analyses furnish an explanation for this growth, because the medium used was not nitrogen-free as supposed. The sugar used was a commercial product, thus accounting for the presence of the nitrogen.

The data presented in Tables 1, 2, and 3 are typical of a large number of determinations made under very similar conditions. It has been the practice over a period of about five years to include in practically every experiment a check consisting of Ashby's nitrogen-free medium containing various sugars and inoculated with some strain of *Rhizobium*. In all, about 30 strains have been tested in this way. In no instance has any appreciable growth been observed, other than gum formation, nor have any increases in nitrogen been found that could safely be considered greater than experimental error. Under exactly the same conditions flasks were frequently included which contained the same medium except for the addition of a suitable source of nitrogen, and in most cases at least fairly good growths were secured. Approximately 700 culture flasks containing a wide variety of nitrogen-free media, inoculated with numerous strains of nodule bacteria, and incubated at various temperatures, were discarded without analysis because the many analyses made agreed in showing that there is no need to analyze cultures which show but little growth. Assuming that other conditions are satisfactory, the growth of *Rhizobia* is roughly propor-

tional to the quantity of available nitrogen present, that is, up to the point where its nitrogen-hunger is largely appeased.

NITROGEN-FIXATION STUDIES WITH SOYBEAN BACTERIA GROWN ON VARIOUS MEDIA

In the studies reported in this paper an attempt was made to include experiments which covered as many variables as possible in the hope that under some given set of conditions the nitrogen-fixing powers of the nodule bacteria might be demonstrated. Naturally the medium received the greatest attention. Some of the data which bear upon this factor are given below.

Five media which had been recommended for the growth of the organism by some research worker and which seemed best adapted for use in fixation experiments were chosen. A description of these, together with the final analyses made after 22 days incubation, is given in Table 4. Unfortunately, the soil-extract media were not analyzed by the modified method to include nitrates. Under such conditions most of the nitrate nitrogen present would appear in the results as "fixation" because the nitrate not determined in the control would appear in the analyses of the bacterial cultures after having been converted into organic forms. Very likely the 0.22 mgm. N reported as fixed in medium 5 was really not fixation at all. The other data of Table 4 show no fixation and all variations are easily within experimental error.

TABLE 4.—*Results of nitrogen determinations on various media inoculated with soybean bacteria media and incubated 22 days*

Medium No.	Composition of medium in grams per liter	Growth observations	N	Average N	N fixed
			Mgm.	Mgm.	Mgm.
1	{ Hot water extract of 14 gm. sweetclover plants (tops and roots) and dipotassium phosphate, 0.5 gm., sucrose, 10 gm., adjusted to neutrality.	{ Good	{ 1.48 1.45 1.56 1.41 1.46 1.44	{ 1.47 1.49 2.45	{ 0.02
2	{ Hot water extract of 50 gm. soil and sucrose 10 gm.	{ Fair	{ 1.44 .44 .44	{ 2.44	{ —, .01
3	{ Lipman's modified Radicicola solution: Sucrose, 10 gm., dipotassium phosphate, 2 gm.; magnesium sulphate, 0.1 gm.; sodium chloride, 0.5 gm.	{ Slight	{ 1.05 1.07 .08 .11	{ .06 .10	{ .04
4	{ Lipman's Radicicola solution: Sucrose, 10 gm.; dipotassium phosphate, 1 gm.	{ Slight	{ .08 .08	{ 1.06 .08	{ .02
5	{ Joshi's soil extract; Hot water extract of 250 gm. soil; mannitol, 10 gm.; dipotassium phosphate, 0.5 gm.	{ Fair	{ 12.76 12.78 3.01 2.96	{ 2.77 2.99	{ .22

¹ Controls, uninoculated.

² No allowance made for nitrates in soil.

NITROGEN-FIXATION STUDIES ON VARIOUS CARROT-EXTRACT MEDIA INOCULATED WITH NODULE BACTERIA

It is a well-known fact that many plant extracts, both legume and nonlegume, when present in media greatly increase the growth of Rhizobia. The writer (1) found that carrot extract was one of the best for this purpose. It was decided, therefore, to select several

media which have been recommended for the growth of these bacteria and modify them by adding carrot extract to each. This would, of course, give media of fairly high nitrogen content but also media that would, presumably, show very excellent growths. Since previous results had shown no fixation in media low in nitrogen it was desired to determine if fixation would occur in nitrogen-rich media, as many workers have reported. The composition of the media as used, together with the analyses at the end of 25 days, are given in Table 5.

TABLE 5.—Results of nitrogen determinations on cultures of legume bacteria grown on different media, with the addition of carrot extract, and incubated 25 days

Medium No.	Composition of medium in grams per liter	Culture	Growth observations	N	N fixed
				<i>Mgm.</i>	<i>Mgm.</i>
1	Löhnis (modified): Carrot extract, dipotassium phosphate, 0.5 gm.; mannit, 10 gm.; CaCO ₃ in excess.	Red clover	Very good	5.60	—0.22
		Kidney bean	Good	5.43	— .39
		Garden bean	do	5.49	— .33
		Trifolium agarium	do	5.49	— .33
2	Moore's synthetic (modified): Carrot extract, sucrose, 10 gm.; dipotassium phosphate, 1 gm.; magnesium sulfate, 0.2 gm.; tap water, reaction not adjusted.	Red clover	No growth	5.80	— .02
		Kidney bean	Fair	5.60	— .22
		Garden bean	Good	5.72	— .10
		Trifolium agarium	do	5.66	— .16
3	Same as medium 2 except adjusted to 4, Fuller's scale.	Red clover	do	5.90	— .08
		Kidney bean	do	5.72	— .10
		Garden bean	do	5.72	— .10
		Trifolium agarium	do	5.83	— .01
4	Mazé's (modified): Carrot extract, sucrose 20 gm.	Red clover	Fair	5.90	— .08
		Kidney bean	Slight	5.62	— .20
		Garden bean	No growth	5.71	— .11
		Trifolium agarium	Fair	5.63	— .19
5	Lipman's Radiclecola solution (modified): Carrot extract, sucrose 10 gm., dipotassium phosphate 2 gm., MgSO ₄ , 0.1 gm., NaCl, 0.5 gm.	Red clover	Slight	5.64	— .18
		Kidney bean	Fair	5.76	— .06
		Garden bean	Good	5.76	— .06
		Trifolium agarium	do	5.59	— .23
6	Lipman's Radiclecola solution (modified): Carrot extract, dipotassium phosphate, 1 gm., tap water.	Red clover	Fair	5.57	— .25
		Kidney bean	Good	5.55	— .27
		Garden bean	Very good	5.40	— .42
		Trifolium agarium	Good	5.51	— .31
7	Joshi's (modified) medium: Carrot extract, mannit, 10 gm., dipotassium phosphate, 1 gm., tap water.	Red clover	do	5.62	— .20
		Kidney bean	do	5.67	— .15
		Garden bean	Very good	5.50	— .32
		Trifolium agarium	Good	5.76	— .06
8	Ashby's (modified) medium: Carrot extract, dipotassium phosphate, 0.2 gm., NaCl, 0.2 gm., MgSO ₄ , 0.2 gm., CaSO ₄ , 0.1 gm., CaCO ₃ , 1 gm., sucrose, 20 gm.	Red clover	Fair	5.59	— .23
		Kidney bean	Good	5.57	— .25
		Garden bean	Very good	5.44	— .38
		Trifolium agarium	do	5.57	— .25
9	Same as medium 8 except for addition of MnSO ₄ , 0.02 gm., and FeCl ₃ , 0.00025 gm.	Red clover	Fair	5.50	— .32
		Kidney bean	Good	5.50	— .32
		Garden bean	Very good	5.53	— .29
		Trifolium agarium	Good	5.55	— .27
	Control, uninoculated			{ 5.80 5.79 5.85 5.84 }	5.82

Very little comment on the analyses is necessary. With all four strains of the legume organism good growths were secured on practically all of the media and yet no fixation was obtained. Most of the analyses show slight losses rather than gains. No explanation is offered for this except to state that variations, such as those found, or even greater, are very common in the case of Kjeldahl analyses on plant extracts. The growing of bacterial cultures usually increases the variations.

NITROGEN-FIXATION STUDIES WITH RED-CLOVER BACTERIA GROWN IN HEATED AND UNHEATED CLOVER EXTRACT

The assumption has frequently been made that legume bacteria fix nitrogen by means of enzymes. If the host plant should secrete the enzymes rather than the bacteria, it would be expected that the use of the unheated extract of the host plant in culture media might favor fixation by the bacteria growing independent of the higher plants. This experiment was planned, therefore, to determine if there is any difference in the growth or fixation, if any, of the nodule bacteria growing in the heated and unheated juices of the host plant.

About 400 gm. of the whole red-clover plant, including the roots and nodules, were ground in a meat chopper, water added, and the mass filtered. A sterile Pasteur-Chamberland filter was then used to refilter the solution in order to sterilize it without heating. The filtrate was incubated for a few days and found to be sterile, about 650 c. c. of the sterile diluted plant juice being obtained. Half of this was then autoclaved at 15 pounds pressure for 20 minutes and the two portions used for the experiment reported in Table 6.

TABLE 6.—Results of nitrogen determinations on cultures of red-clover bacteria grown in heated and unheated clover-extract media

Medium and treatment	Quantity of extract	Incubated 19 days			Incubated 37 days	
		Growth observations	N	N fixed	N	N fixed
			<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
Control, uninoculated	50		35.81			
	50		36.25			
Without clover extract	0	Slight	.04	0.04	0.08	0.08
	5	do	.05	.05	.15	.15
Clover extract, unheated	5	Fair	3.68	.08	3.79	.19
	10	do	7.40	.19	7.26	.05
	20	Good	14.42	.01	14.77	.36
	50	Very good	37.13	1.10	36.75	.72
Clover extract, heated	5	Fair	3.59	— .01	3.74	.14
	10	do	7.34	.13	7.42	.21
	20	Good	14.59	.18	14.70	.29
	50	Very good	36.65	.62	37.12	1.09

The data show no difference in growth between the heated and unheated media, both producing excellent viscous growths, roughly agreeing with the quantity of plant extract added. The analyses show no gains in nitrogen worthy of note. It is true that with the largest quantity of plant extract added the analyses show an average of about 0.9 mgm. of nitrogen increase, but considering that in this case a large quantity of nitrogen was present, the 0.9 mgm. is scarcely significant. Since these results were obtained the writer has had occasion to make many analyses of plant extracts and is thoroughly convinced that a variation of 1 mgm. nitrogen is not significant where as much as 35 mgm. N is present. Duplicate analyses commonly agree more closely than this for uninoculated material, but not after a considerable portion of the nitrogen in the medium has been converted into miscellaneous forms as a result of bacterial growth. It seems safe to conclude that probably no nitrogen fixation took place in this experiment, particularly in view of the fact that it was only in those flasks which contained perhaps five times as much combined nitrogen as the bacteria needed that the small increases were secured.

NITROGEN-FIXATION STUDIES WITH RED-CLOVER ORGANISM GROWING ON A SAND SLOPE

An experiment was next started to test the rather generally accepted idea that Rhizobia fix more nitrogen if the small quantity fixed is constantly removed, as is commonly believed to occur in the nodule. In order to do this a glass tube about 1.5 inches in diameter and 15 inches long was fitted with rubber stoppers, the lower containing one hole and the upper two holes, through which glass tubes passed. This was filled about one-third full of quartz sand, the tube being placed in a slightly elevated position with the sand extending from end to end. After sterilization, a culture of red-clover bacteria was introduced and the apparatus kept at room temperature. Through one of the two tubes at the upper end was constantly sucked a very slow stream of air which passed over the sand rather than through it. To the other tube was attached connections for delivering Ashby's nutrient solution, containing 1 per cent mannitol. Twice daily during the course of the experiment 25 to 50 c. c. of the nutrient solution was allowed to run on to the upper part of the sand layer. Three liters of this solution were used, analyses for total nitrogen being made on each 750 c. c. portion as it came through, and finally of the sand. The analyses showed gains of 0.02, 0.07, 0.14, and 0.27 mgm. N, respectively, for the four portions, or a total of 0.5 mgm. The analyses of the sand showed no nitrogen. Practically no growth, outside of a trace of gum formation, was observed during the 25 days duration of the experiment. This was to be expected for a nitrogen-free medium in case no nitrogen was fixed.

The experiment was repeated, using Ashby's medium containing 1 per cent sucrose instead of dextrose. The analyses made at three intervals showed -0.10, 0.37, and 0.20 mgm. N fixed and no nitrogen in the sand. Considering the bulk of solution used and the general procedure followed, the results would seem to be within experimental error and to indicate further that the organism probably does not fix much, if any, nitrogen independent of the host.

NITROGEN-FIXATION STUDIES IN CARROT EXTRACT BY STRAINS OF RHIZOBIA

Since previous experiments had shown that the legume bacteria grow well only when in a medium containing fixed nitrogen, particularly certain plant extracts, it seemed desirable to run additional experiments using such media. For these experiments, therefore, a carrot-extract medium was selected. This was prepared by boiling the roots, after passing them through a meat chopper, for a half hour in 2 parts of water to 1 of carrots. The extract was filtered and the clear filtrate added to 100 c. c. portions of Ashby's medium, at the rates of 5 and 25 c. c. The Ashby's solution in this case contained 2 per cent sucrose. After sterilization, different culture flasks were inoculated with 20 cultures of nodule bacteria. In order to insure that conditions were optimum for growth and fixation, two flasks were also inoculated with *Azotobacter vinelandii*. Because of the large number of cultures used, duplicate inoculations were not made. After incubation for 14 days, analyses for total nitrogen were made, these being given in Table 7.

TABLE 7.—Results of nitrogen determinations on cultures of *Rhizobium* grown on Ashby's medium containing 2 per cent sucrose and varying quantities of carrot extract and incubated for 14 days

Culture	5 c. c. carrot extract added per 100 c. c.			25 c. c. carrot extract added per 100 c. c.		
	Growth observations	N	N fixed	Growth observations	N	N fixed
		Mgm.	Mgm.		Mgm.	Mgm.
Kidney bean	Fair	2.12	—0.08	Very good	10.94	—0.06
Garden pea	do	2.27	.07	do	11.68	.08
Lima bean	do	2.17	—03	Good	10.69	—31
Garden pea	Slight	2.06	—14	do	10.47	—53
Cowpea	Fair	2.30	.10	Fair	10.74	—26
Crimson clover	do	2.40	.20	Very good	11.24	.24
Soybean	do	2.32	.12	Fair	10.69	—31
Cowpea	do	2.19	—01	do	10.78	—22
Red clover	Good	2.15	—05	Very good	10.89	—11
Sweet clover	Fair	2.14	—06	do	11.27	.27
Vetch	do	2.12	—08	do	10.36	—64
Trefoil	Good	2.15	—05	do	11.03	.03
Sulla	Fair	2.11	—09	do	10.47	—53
Japan clover	Good	2.14	—06	Good	10.78	—22
Alfalfa	Fair	2.20	.0	do	10.75	—25
Soybean	do	2.15	—05	do	10.43	—57
do	do	2.13	—07	Very good	10.40	—60
Velvet bean	do	2.23	.03	Good	10.96	—04
Garden bean	do	2.20	.0	Very good	10.98	—02
Yellow clover	Good	2.25	.05	do	11.53	.53
<i>Azotobacter vinelandii</i>	do	8.42	6.22	do	19.32	8.32
Control uninoculated		2.33			10.96	
do		2.07			11.63	

It will be observed that regardless of the fact that nearly all of the cultures of the legume organisms made good growths on the medium used, there is little or no indication that any nitrogen fixation took place. Decreases were more common than increases. These decreases probably do not represent actual losses of nitrogen but merely that the bacterial growths so changed the form of nitrogen present that the Kjeldahl method failed to get all of it. This is common when dealing with plant extracts, and again merely emphasizes the fact that any gains obtained on plant extract or similar media must be relatively large if the data are to be given serious consideration so far as proving that the bacteria can use atmospheric nitrogen.

The nitrogen-fixation rate, as shown in Table 7, was about as expected in the case of *Azotobacter vinelandii*. The fixation would probably have been slightly larger had it not been for the fact that the carrot extract contained considerable nitrogen. The fact that the fixation was 2.1 mgm. N higher where the larger quantity of extract was present shows that the added nitrogen did not greatly interfere with the fixation process. It indicates, further, that the *Azotobacter* used the carbohydrates present in the extract for growth and fixation.

An experiment, similar to the one reported in Table 7, was next carried out using Ashby's medium containing various quantities of carrot extract and sucrose. A culture of nodule bacteria from yellow clover was used for inoculation; also one of *Azotobacter vinelandii* for comparison. The analyses, made after 23 days, are given in Table 8.

TABLE 8.—Results of nitrogen determinations on *Rhizobium* and *Azotobacter vinelandii* cultures grown on Ashby's medium containing varying quantities of sucrose and carrot extract and incubated 23 days

Culture	Additions to Ashby's mineral medium per 100 c. c.		Growth observations	N	Average N	N fixed
	Carrot extract	Sucrose				
	C. c.	Gm.		Mgm.	Mgm.	Mgm.
Yellow clover	10	0	Good	4.27	4.25	—0.08
	10	0	do.	4.23		
	25	0	Very good	10.62	10.63	—0.63
	25	0	do.	10.63		
	10	.5	Good	4.31	4.28	—0.05
	10	.5	do.	4.25		
	25	.5	Very good	10.67	10.63	—0.03
	25	.5	do.	10.58		
	10	1.0	Good	4.24	4.24	—0.09
	10	1.0	do.	4.23		
	25	1.0	Very good	10.33	10.48	—0.18
	25	1.0	do.	10.62		
	10	2.0	Good	4.06	4.20	—0.13
	10	2.0	do.	4.34		
	25	2.0	Very good	10.32	10.50	—0.16
	25	2.0	do.	10.67		
A. vinelandii	10	0	Fair	6.29	6.03	1.70
	10	0	do.	5.76		
	25	0	Good	12.78	14.04	3.38
	25	0	do.	15.29		
	25	2.0	Very good	18.69	19.35	8.69
Control, uninoculated	25	2.0	do.	20.00		
	10	0		4.35	4.33	
	10	0		4.30		
	25	0		10.70	10.66	
	25	0		10.62		

The duplicate analyses check very closely, considering that a considerable quantity of plant-extract nitrogen was present, and again show no fixation of free nitrogen regardless of the fact that the growth was exceptionally good. Under the same conditions *Azotobacter vinelandii* fixed nitrogen at a normal rate.

Considerably more data similar to that discussed could be given if space permitted. All of these figures bring out the fact that it is impossible to secure very closely agreeing analyses of bacterial cultures growing on plant-extract media. A summation of the negative and positive results gives a figure near zero but with a slight difference on the negative side. All of the evidence shows that the legume organism under these conditions does not fix nitrogen but merely uses that present in a combined form in the plant juice for its growth. In fact, the main explanation for the increased growth of nodule bacteria on plant-extract media is the presence of the nitrogen, probably in a particularly suitable form.

NITROGEN-FIXATION STUDIES WITH MEDIA CONTAINING VARIOUS COMBINATIONS OF INGREDIENTS

Numerous experiments conducted during the course of these investigations showed that the presence of carrot extract, soil extract, or asparagine in media was favorable to the growth of the nodule bacteria. In some cases manganese salts seemed to be stimulating. In order to find out if increases in nitrogen occurred in media con-

taining these materials, it was decided to test out various combinations of these constituents added to media in quantities that previous experiments had shown would be favorable. Just prior to starting this experiment a sample of "bios" was obtained from W. H. Eddy and included in the study. The reason for including the bios was the fact that it is known to be an excellent growth promoter for yeast, and, furthermore, chemical studies with plant extracts had indicated that the stimulating properties of these for *Rhizobia* might be due partly to their bios content. The various materials were added to Ashby's solution, containing 1 per cent dextrose, sterilized, and inoculated with red-clover bacteria. The analyses for total nitrogen made at the end of 10 days are given in Table 9.

TABLE 9.—Results of nitrogen determinations of *Rhizobia* cultures grown in Ashby's media containing bios and other additions and incubated for 10 days

Additions made to Ashby's minerals in quantities per 100 c. c. media					Growth observations	N	N fixed
Bios	MnCl ₂	Carrot	Soil	Asparagine			
Mgm.	Mgm.	C. c.	C. c.	Mgm.		Mgm.	Mgm.
0	0	0	0	0	Slight	0	0
0	10	0	0	0	do	0	0
0	0	10	0	0	Fair	3.08	— .09
0	0	0	10	0	do	.22	— .02
.25	0	0	0	0	Slight	0	0
0	0	0	0	23	Fair	4.37	— .08
0	10	10	0	0	do	3.20	— .03
0	10	0	10	0	do	.24	0
.25	10	0	0	0	Slight	.64	.04
0	10	0	0	23	Fair	4.48	.03
0	0	10	10	0	Good	3.38	— .03
0	0	10	0	23	do	7.44	— .18
.25	0	0	10	0	Fair	.25	.01
0	0	0	10	23	do	4.47	— .22
0	10	10	10	0	Good	3.56	.15
0	10	10	0	23	do	7.49	— .13
.25	10	0	10	0	Fair	.25	.01
0	10	0	10	23	do	4.53	— .16
.25	10	0	0	23	do	4.32	— .13
0	0	10	10	23	Good	7.66	— .20
.25	0	0	10	23	Fair	4.47	— .22
0	10	10	10	23	Good	7.65	— .21
.25	10	16	10	23	do	7.61	— .25
10	0	0	0	23		4.45	
0	0	0	0	23		4.44	
0	0	10	0	0		3.19	
0	0	10	0	0		3.15	
0	0	0	10	0		.24	
0	0	0	10	0		.24	

¹ The last 6 cultures were uninoculated.

It will be observed that the growth varied directly with the quantity of nitrogen present in the medium, there being practically no growth in a nitrogen-free medium, as is always the case with legume bacteria. No fixation occurred in any of the media, all of the results being within experimental error. Aside from nitrogen fixation the results show, further, that Eddy's bios was without appreciable effect, whether tested in the presence or absence of available nitrogen. Practically the same can be said for manganese chloride under the conditions used. Soil extract, asparagine, and carrot extract all increased the growth, due chiefly to their nitrogen content.

NITROGEN-FIXATION STUDIES WITH RED-CLOVER BACTERIA GROWN IN SPINACH EXTRACT

Among the plant extracts which previous work (1) had shown to be especially good in promoting growths of *Rhizobia* was spinach. An experiment was therefore started to determine if this increased growth meant a fixation of atmospheric nitrogen. Both hot and cold water extracts were prepared, the hot-water extract being made by passing 215 gms. of green spinach through a meat chopper, adding water, boiling one hour, filtering, and making up to 1,300 c. c. The cold-water extract was prepared in the same manner except for the absence of heat. These extracts were added in various proportions to Ashby's medium with and without calcium carbonate and sucrose. The sterilized media in 300 c. c. culture flasks were inoculated with a pure culture of red-clover bacteria and allowed to incubate for 18 days at 28° C. before analysis. The results are given in Table 10.

TABLE 10.—Results of nitrogen determinations on cultures of red-clover bacteria grown on Ashby's medium containing spinach extract, and other additions and incubated 18 days at 28° C.

Additions to Ashby's medium in quantities per 100 c. c. media			Growth observations	N	N fixed
Spinach extract	CaCO ₃	Sucrose			
	Gm.	Gm.		Mgm.	Mgm.
0	0.1	1.0	Slight	0.09	0.09
5 c. c. cold	.1	1.0	Fair	1.48	.08
10 c. c. cold	.1	1.0	do	2.74	-.06
25 c. c. cold	.1	1.0	Good	6.72	-.29
50 c. c. cold	.1	1.0	Very good	13.26	-.76
75 c. c. cold	.1	1.0	Doubtful	20.91	-1.99
100 c. c. cold	.1	1.0	do	25.51	-2.41
5 c. c. hot	.1	1.0	Fair	1.17	.14
10 c. c. hot	.1	1.0	do	2.18	.17
25 c. c. hot	.1	1.0	Good	5.50	.57
50 c. c. hot	.1	1.0	Very good	9.63	-.17
75 c. c. hot	.1	1.0	do	14.59	-.08
100 c. c. hot	.1	1.0	Good	18.02	-1.58
25 c. c. cold	.1	0	Doubtful	5.90	-1.11
25 c. c. cold	.1	0	do	5.86	-1.15
5 c. c. hot	0	1.0	Fair	1.14	.11
10 c. c. hot	0	1.0	do	2.16	.15
25 c. c. hot	0	1.0	Good	5.28	.35
50 c. c. hot	0	1.0	Very good	10.03	.23
75 c. c. hot	0	1.0	do	15.13	.46
100 c. c. hot	0	1.0	do	19.83	.29
25 c. c. hot	0	0	Fair	4.53	-.37
0 ¹	.1	1.0		0	
50 c. c. hot	0	0		9.87	
50 c. c. hot	0	0		9.73	
50 c. c. cold	0	0		14.02	
50 c. c. cold	0	0		14.02	

¹ The last 5 cultures were uninoculated.

These data furnish little evidence that the nodule bacteria can use free nitrogen. A few slight gains in nitrogen were obtained but the losses were considerably greater. Evidently the bacteria obtained all of the nitrogen needed from the plant extract.

COMPARISON OF MEDIA FOR THE GROWTH OF VARIOUS STRAINS OF RHIZOBIA

An experiment to compare nitrogen fixation, if any, by *Rhizobia* grown in solution and on agar, using three media, was started. The solution cultures were grown in 100 c. c. portions of media in 500 c. c. Erlenmeyer flasks for a period of 39 days. The agar cultures were

made in Petri dishes, but were not analyzed because of the numerous fungus contaminations which occurred. The solution cultures were analyzed for total nitrogen, however, and these data are given in Table 11.

TABLE 11.—*Results of nitrogen determinations on Rhizobia cultures grown on various solution cultures and incubated for 39 days*

Medium	Culture	Growth observations	N	Average N	N fixed
			Mgm.	Mgm.	Mgm.
Greig-Smith's with CaCO_3 Dextrose 2 per cent Na_2HPO_4 , 0.2 per cent CaCO_3 0.5 per cent.	Red clover	Slight	0.34	0.46	0.15
	do.	do.	.58		
	Soybean	do.	.37	.38	.07
	do.	do.	.38		
	Alfalfa	do.	.40	.40	.09
	do.	do.	Lost.		
Ashby's with 2 per cent dextrose.	Sulla	do.	.36	.36	.05
	do.	do.	Lost.		
	Red clover	Fair	.33	.35	.01
	do.	do.	.36		
	Soybean	do.	.41	.44	.10
	do.	do.	.47		
Ashby's with 2 per cent dextrose, plus spinach extract.	Alfalfa	do.	.39	.39	.05
	do.	do.	.38		
	Sulla	do.	.36	.35	.01
	do.	do.	.34		
	Red clover	Good	12.05	13.09	.27
	do.	do.	13.23		
Control, uninoculated; Greig-Smith's.	Soybean	Slight	Lost.		
	do.	do.			
	Alfalfa	Fair	12.52	12.50	— .32
	do.	do.	12.48		
	Sulla	Good	13.12	12.80	— .02
	do.	do.	12.48		
Control, uninoculated; Ashby's.			.28	.31	
			.34		
			.34	.34	
			.34		
			13.01		
			12.77		
Control, uninoculated; Ashby's with spinach.			12.77	12.82	
			12.74		

The results merely substantiate data previously given and show no fixation by any of the four cultures of *Rhizobia* grown on the three media, the variations being within experimental error. Duplicate determinations failed to agree quite as closely in this experiment as in most of the others given in this paper, but the variations between duplicates were in only one case greater than 0.28 mgm. nitrogen. Unfortunately four of the determinations were lost due either to breakage of flasks or contamination of cultures.

USE OF VARIOUS CARBOHYDRATES BY RHIZOBIA

Most of the experimental work with legume-nodule bacteria, reported above, was conducted in nutrient media containing dextrose as the source of energy. In some cases miscellaneous carbohydrates, found in various plant juices, have also been present in varying percentages. In other experiments, not reported here, the growth of the organism on other sugars, including the hydrolyzed products of starches, gums, cellulose, and miscellaneous materials, has been determined. Practically all of these mixtures served as suitable energy sources but seemed to be no better than any one of the four sugars commonly used, namely, sucrose, dextrose, mannitol, and maltose. As an additional check on this, and also in order to determine if nitrogen fixation occurs with any of the carbohydrates, the experiment reported in Table 12 was conducted. Four media were used: (1) Ashby's minerals with the various energy sources, (2) Ashby's

with 0.05 per cent potassium nitrate for the purpose of serving as a check on growth, (3) Ashby's with 0.2 per cent egg albumen, and (4) Ashby's with 0.2 per cent egg albumen and 0.05 per cent potassium nitrate. The reason for the use of the albumen was that in rather extensive studies, not yet reported, it was found that a certain commercial egg albumen served to increase the growth of certain strains of *Rhizobia*, provided an available form of nitrogen was also present in the medium. In the absence of the fixed nitrogen the albumen had very little effect. It seemed, therefore, that this would be an ideal method of testing the organism for nitrogen-fixing powers and far superior to the use of a plant extract of variable composition. In Ashby's solution containing certain brands of commercial egg albumen and nitrogenous salts the conditions for the growth of some strains of *Rhizobia* seem to be nearly ideal and in many cases superior to plant-extract media. It was further determined that the egg albumen was not used to any appreciable extent, if at all, by the bacteria. Apparently either the impurities present or the physical properties of the albumen were responsible for the beneficial effects on bacterial growth. By omitting all available forms of nitrogen it would seem that conditions would be ideal for fixation if the organism possesses this ability. As a check on the culture work a series of flasks was also included in which both egg albumen and potassium nitrate were present. The cultural methods used were the usual ones, the culture being a red-clover organism which was grown in 100 c. c. of the various media containing 1 per cent of the carbohydrate. After incubation at 28° C. for a period of 65 days the egg albumen series was analyzed for total nitrogen. Analyses were not made on the series containing Ashby's nitrogen-free medium because the growth was almost negligible in all cases. Neither were analyses made on the other two series, containing nitrogen, carried along largely for comparison. The observations and data are given in Table 12.

TABLE 12.—Results of nitrogen determinations on cultures of the red-clover strain of *Rhizobia* grown on different media in the presence of various carbohydrates

Carbohydrate	Ashby's nitrogen-free medium, growth observations	Ashby's medium with 0.05 per cent KNO_3 , growth observations	Ashby's medium with 0.2 per cent egg albumen			Ashby's medium with 0.2 per cent egg albumen and 0.05 per cent KNO_3 , growth observations
			Growth observations	N	N fixed	
				<i>Mgm.</i>	<i>Mgm.</i>	
Dextrose, commercial.	Slight	Good	Fair	25.00	0.19	Very good.
Sucrose, commercial.	do	do	do	25.09	.28	Do.
d-Mannitol, c. p.	do	do	do	24.96	.15	Do.
Lactose, c. p.	do	Fair	do	24.65	-.16	Do.
d-mannose, c. p.	do	do	do	24.96	.15	Do.
l-arabinose, c. p.	do	Slight	do	24.73	-.08	Good.
l-xylose, c. p.	do	do	do	24.52	-.29	Do.
d-galactose, c. p.	do	Fair	do	24.70	-.11	Do.
Raffinose, c. p.	do	do	Slight	24.96	.15	Do.
Maltose, c. p.	do	Contaminated	Fair	24.96	.15	Very good.
d-glucose, c. p.	do	Fair	do	25.09	.28	Do.
Glycerine, c. p.	do	do	do	24.79	-.02	Do.
Dextrin, commercial.	do	do	do	Lost.		Do.
Soluble starch (Lintner).	No growth	No growth	No growth			No growth.
Starch, commercial.	do	do	do			Do.
Gum arabic.	do	do	do			Do.
Control for analysis.				24.89		
Do.				24.59		
Do.				24.95		

The results of the analyses were all within experimental error, with the slight apparent gains in a few instances approximately balancing the slight losses obtained in others. With conditions ideal for growth, except for the absence of a supply of available nitrogen, no fixation occurred. All of the carbohydrates used, with the exception of starch and gum arabic, apparently served as suitable sources of energy. This statement is, however, based on observations only, since no sugar determinations were made.

NITROGEN-FIXATION STUDIES WITH LEGUME-NODULE BACTERIA GROWING ON AGAR

In some of the investigations which have been reported in the literature showing nitrogen fixation by *Rhizobium*, the cultures were grown on agar media. In general, the observations indicated that the growth was better under such conditions than where the organisms were in solution cultures, and the fixation in some instances was reported as greater. These findings would seem to be logical in case the organism fixes nitrogen, because on the host plant the organisms are not submerged in water. The root nodules are normally exposed to the soil air; in fact, when submerged for any great length of time they usually cease to function, or at least fixation proceeds at a slower rate. The writer has frequently observed that the organisms on agar cultures often seem to grow much better in the laboratory than do those in solution cultures, but this may be merely because the organisms and the gum are more apparent on agar. Certainly the organisms die out sooner in solution.

A preliminary experiment was started, which need not be reported in detail here, using three media and four cultures of *Rhizobia*. These cultures were made in Petri dishes and also in some large flat-bottomed culture dishes, similar in shape to Petri dishes. A large number of these became contaminated with fungi before the end of the incubation period. The uncontaminated cultures, practically all of which showed excellent growth with good gum formation, were analyzed for total nitrogen and the results were so near those for the uninoculated control as to be within the limits of experimental error.

Another experiment was then started, using Ashby's medium to which was added 15 gm. of dextrose and 10 gm. of agar per liter. Three-liter Erlenmeyer flasks were used as culture flasks, each containing 100 c. c. of the medium. A culture of alfalfa bacteria was used for inoculation. After incubation for 48 days at room temperature, total nitrogen determinations were made. These are given in Table 13.

TABLE 13.—*Results of nitrogen determinations on cultures of alfalfa bacteria grown on a solid agar medium and incubated at room temperature for 48 days*

Culture	Growth observations	N	Average N	N fixed
		Mgm.	Mgm.	Mgm.
Control, uninoculated.....		<div>2.73</div> <div>2.84</div> <div>2.73</div> <div>2.69</div> <div>2.82</div>	2.75	
Alfalfa bacteria.....	Good...	<div>2.84</div> <div>2.96</div>	2.87	0.12

The average gain in nitrogen was in this case 0.12 mgm. N, which is not enough to be significant. It seems that the organism was able to secure enough nitrogen from the agar to make a rather good growth. The actual mass of bacterial cells produced was probably much smaller than might be expected from a casual glance at the gummy cultures.

A repetition of the experiment, reported above, was then started, using five cultures of legume bacteria. The medium used was Ashby's with and without calcium carbonate at the rate of 2 gm., dextrose 20 gm., and agar 10 gm. per liter. Three-liter flasks, containing 100 c. c. of the above media, were used as culture flasks. These were kept at room temperature for 33 days and analyses then made for total nitrogen. These figures are given in Table 14.

TABLE 14.—Results of nitrogen determinations on cultures of several strains of nodule bacteria grown on an agar medium and incubated at room temperature for 33 days

Culture	Calcium carbonate added	Growth observations	N	Average N	N fixed
	Grams per 100 c. c.		Mgm.	Mgm.	Mgm.
Control, uninoculated	0.2 0 0		3.03 2.98 3.04	3.02	
Red clover	.2 0	Good Fair	2.86 3.04 2.94	2.95 2.94	-0.07 -.08
Sweetclover	.2 0	Good Fair	2.86 2.93 2.91	2.90 2.93	-.12 -.09
Soybean	.2 0	do. do.	3.04 2.94	2.98 2.94	-.04 -.08
Alfalfa	.2 0	Good do.	2.93 3.04 2.98	2.99 2.98	-.03 -.04
Winter pea	.2 0	Fair do.	2.93 3.00	2.93 3.00	-.10 -.03

The conclusions to be drawn from these results are essentially the same as from Table 13. The analyses checked exceptionally closely, the variations between the extremes being only 0.18 mgm. N. Certainly these results are within experimental error and again demonstrate that under the experimental conditions no free nitrogen was fixed by any of the five strains of bacteria. Good growths, considering the medium used, were obtained with three of the cultures and fair growths with the other two. The agar used was a well-purified sample, but presumably the bacteria were able to obtain some available nitrogenous material from it.

As a further test of the nitrogen-fixing power of nodule bacteria another experiment was conducted, using Ashby's mineral solution with the addition of 1 per cent mannitol and 1 per cent agar. This medium was added to 3-liter Erlenmeyer flasks at the rate of 200 c. c. each and sterilized. Ten pure cultures of nodule bacteria were used for inoculation; also a culture of *Azotobacter vinelandii* for comparison. The cultures were incubated at 28° C. for 32 days, after which analyses were made by the Kjeldahl method, using mercury as a catalyst in digestion and sodium thiosulphate for the precipitation of the mercury prior to distillation. Table 15 gives the growth observations and final analyses.

TABLE 15.—Results of nitrogen determinations on cultures of *Rhizobia*, and of *Azotobacter vinelandii*, grown on thin agar layers and incubated at 28° C. for 32 days

Culture	Growth observations	N	Average N	N fixed
		Mgm.	Mgm.	Mgm.
Soybean.....	Fair, very gummy.....	3.67 3.56 3.64 3.49	3.62	0.09
Clover.....	Good, very gummy.....	3.42 3.56 3.51	3.49	— .04
Pea.....	Fair.....	3.40 3.50 3.58	3.47	— .06
Kidney bean.....do.....	3.58 3.58 3.37	3.51	— .02
Alfalfa.....	Good.....	3.51 3.44	3.48	— .05
Lespedeza.....	Slight.....	3.51 3.44	3.48	— .05
Velvetbean.....	Fair.....	3.50 3.63	3.57	.04
Dalea.....	Good.....	3.62 3.54	3.58	.05
Sulla.....do.....	3.40 3.67	3.54	.01
Sweetclover.....	Fair.....	3.39 3.59	3.49	— .04
A. vinelandii.....	Very good.....	21.20 17.36 3.65	19.28	15.75
Control, uninoculated.....		3.51 3.54 3.50 3.46	3.53	—

The organisms made good growths in most cases, considering that they were grown under nitrogen-starvation conditions. The term "good" as used in the table may be misleading, however. In no case was a growth obtained comparable to those ordinarily secured in a medium containing adequate nitrogen. In this case good simply means that the organisms produced a glistening layer of gum on the surface of the agar. Actually the organisms were not making an abundant growth such as would be produced on a suitable plant extract or similar medium.

The evidence in Table 15 shows very conclusively that no nitrogen was fixed by any of the 10 strains of *Rhizobia*. All results were within experimental error. On the other hand, *Azotobacter vinelandii* fixed an average of 7.87 mgm. N per gram of mannit. This is exceptionally high and is the largest fixation per unit of sugar ever secured by the writer with an *Azotobacter* culture. The fact that the medium and growing conditions were so exceptionally favorable for nitrogen fixation makes the negative results with *Rhizobia* seem all the more significant.

NITROGEN-FIXATION STUDIES ON SOIL-EXTRACT MEDIA

A review of the work that has been done with legume-nodule bacteria brings out the fact that these organisms respond in many cases to low concentrations of certain mineral elements not ordinarily added to synthetic nutrient media. Several experiments were made to test out this point with various concentrations of elements, such as Cu, Mn, B, Zn, Pb, etc. There was little to indicate, however, that such elements play an important rôle in nitrogen fixation by nodule bacteria growing in culture flasks. It is true, though, that soil-extract media, which contain traces of many mineral salts, have been reported as favorable to growth and to fixation by the legume

bacteria. Such extracts contain appreciable quantities of nitrogenous salts, which might explain the increased growth effects, but there still seems to be evidence that the mineral constituents present also increase nitrogen fixation by Rhizobia. In fact, soil-extract media have been more or less generally recognized as the best to use in demonstrating the nitrogen-fixing power of these bacteria. Several experiments were, therefore, conducted to determine if the nodule bacteria can fix nitrogen when growing in such media. In all of the experiments which follow the soil extract was prepared by autoclaving the soil for one hour in the presence of a few grams of calcium carbonate and 2 parts of water to 1 of soil by weight. Enough additional water was used to leach out the soil on the filter to make the final weight of soil extract twice that of the original soil.

The medium used in the first experiment consisted of soil extract added in various proportions to a mineral-sugar medium containing 1 per cent mannitol, 0.05 per cent K_2HPO_4 , and 0.1 per cent $CaCO_3$. The experiment was carried out in 500 c. c. Erlenmeyer flasks, each containing 200 c. c. of the soil-extract medium. Six strains of Rhizobia were used for inoculation, the analyses for total nitrogen being made after an incubation period of 21 days. Tests for nitrate nitrogen in the soil extract were made, but the quantity present was too small to affect the Kjeldahl analysis, hence the ordinary method of analysis using K_2SO_4 and $CuSO_4$ was used. The results of these nitrogen determinations, together with the observations of growth, are given in Table 16. Duplicate determinations were not made in this case because of the large number of variables introduced.

TABLE 16.—Results of nitrogen determinations on cultures of nodule bacteria grown on a mineral-sugar medium containing soil extract and incubated 21 days

Culture	Growth observations	Nitrogen content of sterile media	Nitrogen content of inoculated media	Nitrogen fixed
		Mgm.	Mgm.	Mgm.
Red clover	Slight	0	0	0
	Fair	.83	.88	.05
	do.	1.66	1.61	— .05
	Good	3.32	3.14	— .18
	Very good	8.30	8.56	— .26
Crimson clover	do.	16.60	16.52	— .08
	Slight	0	0	0
	do.	.83	.86	.03
	Fair	1.66	1.61	— .05
	do.	3.33	3.52	.19
Alfalfa	Good	8.30	8.65	.35
	do.	16.60	16.72	.12
	Slight	0	0	0
	Fair	.83	.91	.08
	do.	1.66	1.54	— .12
Soybean	Good	3.33	3.28	— .05
	Very good	8.30	8.34	.04
	do.	16.60	16.25	— .35
	Slight	0	.03	.03
	do.	.83	.98	.15
Cowpea	Fair	1.66	1.59	— .07
	do.	3.33	3.44	.11
	do.	8.30	7.82	— .48
	do.	16.60	15.90	— .70
	Slight	0	.07	.07
Vetch	do.	.83	.97	.14
	Fair	1.66	1.64	— .02
	do.	3.33	3.48	.15
	do.	8.30	8.03	— .27
	do.	16.60	16.40	— .20
	Slight	0	.08	.08
	Fair	.83	.89	.06
	do.	1.66	1.35	— .31
	Good	3.33	Lost.	—
	Very good	8.30	7.96	— .34
	do.	16.60	16.17	— .43

The results show that in most cases the growth of the organisms was roughly proportional to the nitrogen content of the medium in which they grew. This was particularly true for the red clover, alfalfa, and vetch strains. The cowpea and soybean cultures made poor growths, as is always the case with these strains. The analyses show no evidence to indicate that any of the strains could fix free nitrogen. A few slight increases in nitrogen were obtained, but in more cases losses were secured. The largest variations were usually where there was more nitrogen present than the bacteria needed. Evidently the stimulating effect of soil extract for nodule bacteria is primarily due to the nitrogen added.

TABLE 17.—Results of nitrogen determinations on cultures of *Rhizobia* grown on a modified Ashby's mineral solution containing various amounts of soil extract

Culture	Quantity of soil extract per 100 c. c.	Incubated 18 days				Incubated 31 days				Average N fixed
		Growth observations	N	Average N	N fixed	Growth observations	N	Average N	N fixed	
			Mgm.	Mgm.	Mgm.		Mgm.	Mgm.	Mgm.	
Red clover	0	Slight	0.06	—0.03	—0.03	Slight	—0.02	—0.03	—0.03	—0.03
	0	do.	—0.13	—0.03	—0.03	do.	—0.04	—0.03	—0.03	—0.03
	5	do.	—0.01	—0.05	—0.14	do.	.08	.10	.01	—0.07
	5	do.	—0.10	—0.05	—0.14	do.	.13	.10	.01	—0.07
	10	Fair	—0.02	.08	—0.10	Fair	.14	.15	—0.03	—0.07
	10	do.	.19	.08	—0.10	do.	.17	.15	—0.03	—0.07
	25	do.	.56	.54	.08	do.	.48	.50	.04	.06
	25	do.	.53	.54	.08	do.	.53	.50	.04	.06
	75	Good	1.52	1.44	.07	Good	1.45	1.45	.08	.08
	75	do.	1.36	1.44	.07	do.	Lost.	1.45	.08	.08
Cowpea	0	Slight	.12	.06	.06	Slight	.07	.03	.03	.05
	0	do.	0	.06	.06	do.	—0.02	.03	.03	.05
	5	do.	.11	.12	.03	do.	.02	—0.04	—0.13	—0.05
	5	do.	.13	.12	.03	do.	—0.09	—0.04	—0.13	—0.05
	10	do.	0	.04	—0.14	do.	.20	.20	.02	—0.06
	10	do.	.08	.04	—0.14	do.	Lost.	.20	.02	—0.06
	25	Fair	.40	.48	.02	Fair	.47	.47	.01	.02
	25	do.	.57	.48	.02	do.	.46	.47	.01	.02
	75	do.	1.52	1.52	.15	do.	1.46	1.44	.07	.11
	75	do.	1.52	1.52	.15	do.	1.41	1.44	.07	.11
Soybean	0	Slight	.02	.01	.01	Slight	—0.01	—0.03	—0.03	—0.01
	0	do.	0	.01	.01	do.	—0.05	—0.03	—0.03	—0.01
	5	do.	.07	.13	.04	do.	.02	.07	—0.02	.01
	5	do.	.19	.13	.04	do.	.11	.07	—0.02	.01
	10	Fair	.35	.35	.17	Fair	.17	.17	—0.01	.08
	10	do.	Lost.	.35	.17	do.	.17	.17	—0.01	.08
	25	do.	.53	.47	.01	do.	.47	.46	0	.01
	25	do.	.40	.47	.01	do.	.44	.46	0	.01
	75	Good	1.27	1.39	.02	Good	1.39	1.43	.06	.04
	75	do.	1.50	1.39	.02	do.	1.47	1.43	.06	.04
Alfalfa	0	Slight	0	.02	.02	Slight	—0.05	—0.07	—0.07	—0.03
	0	do.	.04	.02	.02	do.	—0.08	—0.07	—0.07	—0.03
	5	do.	.04	.02	—0.07	do.	.04	.05	—0.04	—0.06
	5	do.	0	.02	—0.07	do.	.06	.05	—0.04	—0.06
	10	do.	.07	.04	—0.16	do.	Lost.	.08	—0.10	—0.13
	10	do.	0	.04	—0.16	do.	.08	.08	—0.10	—0.13
	25	Fair	.21	.17	—0.29	Fair	.40	.39	—0.07	—0.18
	25	do.	.13	.17	—0.29	do.	.38	.39	—0.07	—0.18
	75	Good	1.56	1.36	—0.01	Good	1.38	1.40	.03	.01
	75	do.	1.15	1.36	—0.01	do.	1.41	1.40	.03	.01
Uninoculated	50						.91			
	50						.89			
	50						.94	.91		

A second experiment dealing with nitrogen-fixing powers of various strains of *Rhizobium* was made in Ashby's mineral solution containing 2 per cent dextrose, 0.05 per cent K_2HPO_4 instead of 0.02 per cent, and various quantities of soil extract. Two-liter Erlenmeyer flasks containing 100 c. c. of the medium were used for the culture work. The soil extract was prepared in the usual manner, except that the soil was first leached with cold water to remove the nitrates. This was done in order to avoid the necessity of using the much longer and more difficult reduction method for including nitrate nitrogen. While this method is accurate, the experimental error is usually larger, due to the longer procedure used. This is especially true where solutions are analyzed which contain materials, such as sugars or calcium carbonate, which cause frothing. Several comparisons of the Kjeldahl method, in which potassium sulphate, copper sulphate, and sulphuric acid were used in the digestion flask with the older method in which metallic mercury and sulphuric acid were used, seemed to give slightly better results on soil-extract media with the latter method. This method of analysis was, therefore, used in this experiment. Sufficient sodium thiosulphate was added prior to distillation to precipitate the mercury. Analyses were made at the end of both 18 and 31 days, the results together with the observations of growth being given in Table 17.

The growth of the four strains of legume bacteria was again roughly proportional to the quantity of nitrogen added in the soil extract. Practically no visible growths were secured on the media containing no soil extract, but fair to good growths were secured with the larger quantities of extract added. Duplicate nitrogen determinations checked unusually well, and the results, in general, show even more conclusively than in previous tables that the nodule bacteria did not fix atmospheric nitrogen. The variations from the control are within experimental error with about an equal number of negative results as positive. In this experiment the medium used was ideal for the growth of *Rhizobia* except for the absence of sufficient fixed nitrogen. Thin layers of solution and large flasks were used to provide excellent aerobic conditions, and yet no fixation was obtained with 4 representative strains of the nodule bacteria.

While the above experiment was in progress 5 additional cultures of root-nodule bacteria were received from E. B. Fred, of the University of Wisconsin, and a third experiment immediately started which was in all essential respects a duplication of that reported in Table 16. Again a soil extract was prepared, using a soil from which the nitrates were first leached with cold water. Two-liter flasks were used, some containing 100 c. c. and others 200 c. c. of the soil-extract medium, according to the scheme shown in Table 18. All tests were made in triplicate with each of the nine strains of *Rhizobia*. The first four of these were the cultures used in obtaining the results reported in Table 17, the other 5 were those received from Fred. After the cultures had been incubated for 41 days analyses were made by the Kjeldahl method, using mercury in the digestion flasks.

TABLE 18.—*Results of nitrogen determinations on cultures of Rhizobia grown on a mineral-sugar medium containing various amounts of soil extract and incubated 41 days*

Culture	Quantity of soil extract media	Growth observations	N	Average N	N fixed
			Mgm.	Mgm.	Mgm.
Red clover.....	100	Good	1.97	2.01	—0.02
			2.04		
	200	do	2.03	3.83	.03
			3.82		
Cowpea.....	100	Fair.....	3.84	2.02	— .01
			2.03		
	200	do	2.00	3.81	.01
			2.03		
Soybean.....	100	do	3.84	2.08	.05
			3.78		
	200	do	2.06	3.82	.02
			3.84		
Alfalfa	100	Slight.....	3.84	2.01	— .02
			1.99		
	200	Fair	2.00	3.80	.00
			3.82		
Clover (1).....	100	do	3.81	2.06	.03
			3.78		
	200	Good.....	2.14	3.81	.01
			2.00		
Clover (2).....	100	Fair	2.03	1.97	— .06
			3.79		
	200	Good.....	3.84	3.83	.03
			1.88		
Dulse.....	100	Fair	2.00	1.98	— .05
			2.02		
	200	Good.....	3.82	3.81	.01
			3.84		
Pea (1).....	100	Fair	3.79	2.04	.01
			2.07		
	200	Good.....	2.03	3.83	.03
			3.82		
Pea (2).....	100	Slight.....	3.84	2.00	— .03
			1.96		
	200	Good	2.00	3.81	.01
			2.04		
Control uninoculated.....	100		3.81	2.03	
			2.02		
	200		2.06	3.80	
			3.84		

The data agree very closely with those given in Table 17, and the conclusions to be drawn are essentially the same.

A fourth experiment, similar in many respects to those reported in Tables 17 and 18 was next started, using an extract of a soil from which the nitrates were not first leached with cold water. However, in order to make unnecessary the use of the reduction method to include nitrates, the soil used was secured from the field in the late winter when only a trace of nitrates was present. The medium contained Ashby's mineral solution with 0.05 per cent K_2HPO_4 , 2 per cent dextrose, and 50 c. c. of soil extract. One hundred c. c. of this was used per 2-liter flask. To a portion of the flasks, as shown in Table 19, were added 750 mgm. agar. This provided for a comparison

of fixation, if any, on liquid and solid media under conditions which provided excellent aeration. The nine cultures of nodule bacteria used in the preceding experiment were also used in this. The analyses were made by the Kjeldahl method, using mercury as a catalyst. In addition, 18 liquid cultures were analyzed by the modified Kjeldahl method, using Devarda's alloy to reduce any nitrates present. This latter series was run largely as a check on the rest of the experiment. As a further check another series of cultures was used for nitrate determinations by the colorimetric method. The analyses made at the end of 53 days are given in Table 19.

TABLE 19.—Results of nitrogen determinations on cultures of various strains of *Rhizobia* grown on modified Ashby's mineral solution, liquid and solid, containing soil extract and incubated 53 days

Culture	Type of culture	Growth observations	Kjeldahl method			Modified Kjeldahl method		
			N	Average N	N fixed	N	Average N	N fixed
			Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.
Red clover	Solution	Good	2.96	2.97	0.01	{ 2.84 2.62 }	2.73	-0.23
			2.97					
	Agar	do.	2.99	4.15	- .17			
			4.15					
Cowpea	Solution	Fair	4.12	2.94	- .02	{ 2.77 2.73 }	2.75	- .21
			4.19					
	Agar	do.	2.97	4.22	- .10			
			2.93					
Soybean	Solution	do.	3.06	3.04	.08	{ 2.62 2.77 }	2.70	- .26
			3.02					
	Agar	do.	4.16	4.21	- .11			
			4.25					
Alfalfa	Solution	do.	4.21	3.02	.06	{ 2.96 3.03 }	3.00	.04
			3.03					
	Agar	Good	2.97	4.24	- .08			
			4.26					
Clover (1)	Solution	Fair	4.21	3.06	.10	{ 2.89 2.98 }	2.94	- .02
			3.06					
	Agar	do.	3.02	4.24	- .08			
			4.25					
Clover (2)	Solution	do.	4.21	3.04	.08	{ 2.75 2.72 }	2.74	- .22
			3.09					
	Agar	Good	3.07	4.28	- .04			
			2.97					
Dalea	Solution	do.	4.26	2.95	- .01	{ 2.90 2.56 }	2.73	- .23
			lost					
	Agar	do.	2.96	4.20	- .12			
			4.12					
Pea (1)	Solution	do.	4.37	2.90	- .06	{ 2.69 2.91 }	2.80	- .16
			2.93					
	Agar	do.	2.88	4.14	- .18			
			4.18					
Pea (2)	Solution	do.	3.97	2.92	- .04	{ 2.55 2.77 }	2.66	- .30
			2.93					
	Agar	do.	2.92	4.00	- .32			
			3.97					
Control, uninoculated	Solution		4.09	2.86		{ 2.94 2.98 }	2.96	
			3.93					
	Agar		2.86	4.25		{ 4.36 4.34 4.27 }	4.32	
			2.88					

The results show a larger number of slight losses of nitrogen than of gains, but the variations from the control are not great enough to be significant. Certainly there is no evidence in these results to indicate that *Rhizobium* can fix nitrogen when grown on either a solid or liquid medium. Qualitative tests for nitrates in the cultures after the end of the growth period showed none present. This agrees with actual analyses by the two methods in showing that the use of the reduction method was not necessary in this case.

GENERAL DISCUSSION

A lengthy discussion of the data presented in the previous pages is entirely unnecessary since the results of all of the experiments agree in showing that the legume bacteria did not fix atmospheric nitrogen when grown under ordinary laboratory conditions independent of the host plant. Of course it would be foolish to say that these bacteria never fix nitrogen under any conditions because obviously no one can test all of the variables. The writer does feel, however, from the data presented that there is little justification for the more or less generally accepted idea that legume-nodule bacteria can fix small quantities of nitrogen when grown independent of the host.

As for the previous work done on this subject, little need be said that was not stated in the historical review. To summarize this phase of the subject, it may be stated that much of the earlier work was done with impure cultures and need not be considered. Furthermore, only a few investigators have made extensive studies of the subject and only a comparatively few papers present as many as 40 analyses. Rarely has an appreciable fixation been reported for cultures grown on nitrogen-free media, and often the more nitrogen added, the greater were the fixations obtained even where more nitrogen was added than the bacteria could possibly have used. The use of soil-extract media has also led to misleading results in some cases due to the presence of nitrate nitrogen. The "official" method using salicylic acid for the determination of total nitrogen, including nitrates, is reliable only in the absence of moisture. Bacteriologists have frequently used it for the determination of total nitrogen in soil extracts. The nitrogen not found by this method appears as "fixation" in the final analysis of the culture after the bacteria have converted the nitrates into proteins.

Since the evidence points very strongly to the fact that legume-nodule bacteria do not fix nitrogen outside of the nodule we naturally wonder if they fix nitrogen in the nodule. We must wait for additional studies for a definite answer to this question. Very likely the host plant is just as important as are the bacteria in the utilization of free nitrogen gas and both the higher and lower plants work in close cooperation in the fixation process.

SUMMARY

This paper presents a rather complete review with a discussion of the literature dealing with nitrogen fixation by legume-nodule bacteria grown independent of the host.

A report of a large number of experiments planned to determine whether legume bacteria possess the power of fixing nitrogen apart from the host is given. These investigations were conducted over a

period of five years, using 31 strains of legume-nodule bacteria, grown in a very large number of media and under a variety of cultural conditions. Practically all of the factors which have been reported as important in demonstrating fixation of nitrogen by these bacteria were given consideration. The experiments in no case gave any evidence that *Rhizobia* can fix atmospheric nitrogen when grown apart from the host. These findings are based on about 600 analyses of cultures reported in this paper, about 300 not reported here, and about 700 additional cultures not analyzed because they showed little or no growth on nitrogen-deficient media.

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EFFECT OF VARIOUS FUMIGANTS ON THE GERMINATION OF SEEDS¹

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Recently several new substances have been developed as fumigants to replace carbon disulphide. In a series of recent publications, Roark and Cotton have discussed the merits of certain aliphatic chlorides,² ethylene dichloride,³ certain alkyl and alkylene formates,⁴ some esters of halogenated fatty acids,⁵ and ethylene oxide.⁶

As some of these substances find commercial application as fumigants, notably ethylene dichloride and the formates, the question arises as to the extent to which they may affect the germination of seeds. In the work reported, Roark and Cotton tested the effect on the germination of wheat, but there was no evidence to show whether all seeds would react in the same way. Hoyt⁷ reported the effect of ethylene dichloride on the germination of wheat, oats, beans, peanuts, and clover, dismissing as of no importance an increase of 24 per cent in the germination of peanuts and a decrease of 21 per cent in the germination of beans.

Although the most extensive use of fumigation is on wheat prior to milling, in which case the effect on germination is not important, the fumigating of seeds stored for the next season's planting is also practiced. In order to obtain further data concerning the effect of the new fumigants, the work here reported was undertaken. Six of the most promising fumigants were tested upon 13 different kinds of seeds, an attempt being made to select those most likely to be fumigated commercially.

Table 1 shows the minimum lethal concentration required to kill rice weevils (*Sitophylus oryza*) buried in wheat and exposed to the action of the fumigant for 24 hours. It shows also the boiling point and the specific gravity of the different fumigants tested. These data are compiled from the publications cited.

¹ Received for publication Apr. 10, 1929; issued December, 1929.

² ROARK, R. C., and COTTON, R. T. FUMIGATION TESTS WITH CERTAIN ALIPHATIC CHLORIDES. *Jour. Econ. Ent.* 21: 135-141. 1928.

³ COTTON, R. T., and ROARK, R. C. ETHYLENE DICHLORIDE-CARBON TETRACHLORIDE MIXTURE; A NEW NONBURNABLE, NONEXPLOSIVE FUMIGANT. *Jour. Econ. Ent.* 20: 636-639. 1927.

⁴ ——— and ROARK, R. C. FUMIGATION OF STORED-PRODUCT INSECTS WITH CERTAIN ALKYL AND ALKYLENE FORMATES. *Indus. and Engin. Chem.* 20: 380-382. 1928.

⁵ ROARK, R. C., and COTTON, R. T. INSECTICIDAL ACTION OF SOME ESTERS OF HALOGENATED FATTY ACIDS IN THE VAPOR PHASE. *Indus. and Engin. Chem.* 20: 512-514. 1928.

⁶ COTTON, R. T., and ROARK, R. C. ETHYLENE OXIDE AS A FUMIGANT. *Indus. and Engin. Chem.* 20: 805. 1928.

⁷ HOYT, L. F. FURTHER FUMIGATION TESTS WITH ETHYLENE DICHLORIDE-CARBON TETRACHLORIDE MIXTURE. *Indus. and Engin. Chem.* 20: 931-932, illus. 1928.

TABLE 1.—*Minimum lethal concentration, boiling point, and specific gravity of various fumigants tested*

[Rice weevils, buried in wheat were exposed to the fumigant for 24-hour periods]

Fumigant	Minimum lethal concentration	Boiling point	Specific gravity
	<i>Mgm. per liter</i>	<i>°C.</i>	
Ethylene oxide.....	30	10.7	0.89
Tertiary butyl chloride.....	34	51.0	.84
Isopropyl formate.....	53	71.3	.88
Methyl chloroacetate.....	73	131.5	1.22
Ethylene dichloride.....	226	83.7	1.26
Trichloroethylene.....	650	88.0	1.48

In carrying out the experiments reported in the present paper 100 gm. of the seed to be tested was placed in a 500 c. c. Erlenmeyer flask, and the fumigant, measured in a 1 c. c. pipette graduated to 0.01 c. c., was allowed to fall directly on the grain. The flasks were stoppered and allowed to stand for 24 hours at room temperature. Two concentrations were used, the minimum lethal concentration and twice that quantity. The results obtained are shown in Table 2.

TABLE 2.—*Percentage germination of different kinds of seeds when untreated and after a 24-hour exposure to various fumigants in different strengths **

Kind of seed tested	Percentage germination of untreated seed	Percentage germination of seed after 24-hour exposure to—											
		Ethylene oxide		Tertiary butyl chloride		Isopropyl formate		Methyl chloroacetate		Ethylene dichloride		Trichloroethylene	
		30 mgm.	60 mgm.	34 mgm.	68 mgm.	53 mgm.	106 mgm.	73 mgm.	146 mgm.	226 mgm.	452 mgm.	650 mgm.	1,300 mgm.
Wheat.....	96	0	0	96	96	95	98	22	2	96	94	96	94
Oats.....	54	0	0	56	59	51	58	50	42	62	53	58	48
Barley.....	98	33	14	99	99	99	97	48	39	98	98	99	98
Rye.....	96	0	0	98	98	98	97	4	29	98	98	98	96
Corn.....	91	0	0	86	79	85	89	13	0	85	85	81	84
Buckwheat.....	67	0	0	67	70	63	64	56	60	62	64	65	56
Sunflower.....	93	12	0	90	88	92	92	92	65	92	92	96	90
Beans.....	91	34	30	93	93	90	92	67	63	86	93	91	93
Lima beans.....	84	0	0	90	84	86	90	76	45	87	88	82	86
Cowpeas.....	27	6	0	58	23	47	35	14	27	42	12	38	28
Alfalfa.....	64	58	57	63	59	60	66	62	66	59	72	60	64
Clover.....	32	10	4	36	34	26	38	30	24	35	32	31	36
Timothy.....	81	0	0	87	79	88	86	0	0	90	86	77	83

* The germination tests were made by the Seed Testing Laboratory, Bureau of Plant Industry; the tests were made in duplicate on 100 seeds.

Of the six fumigants tested, ethylene oxide and methyl chloroacetate were found to be injurious to the germination of grain. With the heavier concentration of ethylene oxide, nine of the varieties of seeds showed no germination whatever, and with the exception of alfalfa, the germination of the others was seriously impaired. The injury from methyl chloroacetate was not so severe, the stronger concentration reducing on an average the percentage of germination to approximately 50 per cent, alfalfa again being an outstanding exception.

The other four fumigants, tertiary butyl chloride, isopropyl formate, ethylene dichloride, and trichloroethylene, were all quite harmless. In no case did the average percentage germination of all 12 kinds of seeds (cowpeas being excluded) vary more than 2 per cent from that of the untreated seed, which was well within the limits of accuracy of the experiment.

The different seeds reacted rather uniformly, except cowpeas and alfalfa. The latter was by far the most resistant of all those tested. The percentage germination of the cowpeas, which was very low in the untreated seed, was stimulated to a remarkable degree by the weaker concentrations of the four fumigants that were not toxic, the average being 46, as against 27 for the untreated seed. It behaved on the whole so erratically and the seed was so poor, being badly infested with weevils, that it was omitted in making up the averages. Corn seemed to be affected somewhat unfavorably by both the weaker and the stronger concentrations of the nontoxic fumigants, its average germination being 84 per cent as against 91 per cent in the untreated seed.

CONCLUSIONS

Wheat, oats, barley, rye, corn, buckwheat, sunflower, beans, Lima beans, cowpeas, alfalfa, clover, and timothy seeds may be fumigated with tertiary butyl chloride, isopropyl formate, ethylene dichloride, and trichloroethylene in concentrations up to twice the minimum lethal concentration required to kill rice weevils without seriously impairing the germination of the seeds.

Ethylene oxide and methyl chloroacetate seriously impair the germination of these seeds.

RELATION OF STOMATAL BEHAVIOR TO STEM-RUST RESISTANCE IN WHEAT¹

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INTRODUCTION

There has been much speculation regarding the nature of resistance of cereals to *Puccinia graminis* Pers. Such accurate observations and experiments as have been made indicate two types of resistance of wheat varieties to *Puccinia graminis tritici* Erikss. and Henn., namely, physiological and morphological.

The so-called physiological resistance appears to be due to some incompatibility between the wheat plant and the pathogene, but its exact nature is yet unknown. The germ tubes of the pathogene may enter physiologically resistant varieties in a perfectly normal manner, but after they have entered they kill some of the plant cells and then they themselves die. A variety, of-course, may be resistant to certain physiologic forms of *Puccinia graminis tritici* and completely susceptible to others. There appears, therefore, to be a high degree of specificity between the host plant and particular physiologic forms. Physiologically resistant varieties may be resistant in certain regions in a given year and susceptible in other regions. Likewise they may be resistant in the same region in one year and susceptible in another. The amount of rust on them depends on the presence or absence of forms which can attack them. For example, Kanred wheat is nearly immune from about a dozen of the physiologic forms so far described and is completely susceptible to many of the others. There is nothing to indicate that Kanred is resistant for any other reason except that of a mutual antagonism between it and those particular physiologic forms to which it is resistant.

Varieties physiologically resistant to certain physiologic forms apparently are equally resistant in all stages of development. It was long supposed that varietal resistance could be explained entirely on the physiologic basis. But it was found that varieties might be susceptible in the seedling stage and at least moderately resistant in later stages of development. This led to the discovery of the morphological type of resistance.

Varieties with the morphological type of resistance may be physiologically susceptible, but there may be structural characteristics which prevent the rust fungus from developing extensively within the tissues. The pathogene may enter the host readily and can develop normally in the chlorophyllous tissues. In the stem the rust can

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grow only in the collenchyma bundles. If these are large and numerous, the organism can grow extensively and produce large pustules. In some varieties, however, there is so much sclerenchyma and the collenchyma bundles of the stem are so small and few that the rust is restricted to relatively small areas. The pustules, therefore, are correspondingly small, and for practical purposes the variety is partially resistant. Kota wheat, for example, is highly susceptible to certain physiologic forms of *Puccinia graminis tritici* in the seedling stage and at least moderately resistant in later stages. Therefore it often is resistant in the field. A variety, of course, may be physiologically susceptible to some physiologic forms, physiologically resistant to others, but morphologically resistant to both.

Still other varieties are susceptible when seedlings and older plants are inoculated and incubated artificially in the greenhouse, but they remain almost free from rust in the field despite the fact that physiologic forms to which they are susceptible in the greenhouse may be present in abundance in the field. When pustules do develop on these varieties in the field, however, they seem to be fairly normal. It appears, therefore, that they have neither physiological nor morphological resistance. It would seem that, although the rust can develop in these varieties when it once enters them, it often is prevented from entering. For example, Velvet Don (C. I.³ 1445), a durum wheat, is very susceptible to form 21 of *Puccinia graminis tritici* when grown in the greenhouse. The fungus readily infects this variety in all stages of development, from the seedling stage until after the plants have headed. Velvet Don evidently has no physiological resistance to form 21. Furthermore, the development of the fungus does not seem to be restricted to any great extent by morphologic peculiarities of the host. The broad and deep collenchyma strands of the culm often coalesce to form wide bands extending lengthwise of the stem and about one-fourth or one-third of the distance around it. Apparently, then, Velvet Don has neither physiologic nor morphologic resistance. Nevertheless, there usually is little or no rust on this variety at University Farm, St. Paul, Minn., even in seasons when *Puccinia graminis tritici* form 21 is very prevalent. The same marked freedom from rust has been noted in other wheat varieties during certain seasons and in certain localities where considerable infection might be expected because of the presence of abundant inoculum and favorable environmental conditions for rust infection and development.

As the germ tubes of aeciospores and urediniospores of *Puccinia graminis tritici* enter wheat plants only through the stomata, it seems that stomatal conditions may account for the inability of the fungus to enter. The stomata are numerous on stem, leaf, rachis, glume, and awn of cereal plants. If they remain closed much of the time, however, it probably would be difficult for the germ tubes to enter under natural conditions in the field. It is quite likely also that the stomata of different varieties may behave quite differently. The writer therefore made a study of stomatal behavior in certain selected varieties of the factors affecting the stomatal movements, and the possible relation of stomatal behavior to the ability of germ tubes to enter.

LITERATURE REVIEW

The relation of stomatal movement to infection of the sugar-beet plant by *Cercospora beticola* was studied by Pool and McKay (11)⁴ in 1916. They found the greatest susceptibility to infection concomitant with the greatest stomatal activity, both occurring on leaves of the same degree of maturity. In detailed studies on germ-tube penetration they found that conidia may germinate and produce long germ tubes and yet not penetrate through closed stomata. Whenever infection occurred the stomata were open. The stomatal activity, in turn, was influenced by light, temperature, and relative humidity.

Cobb (3), as early as 1892, made some interesting observations on the number, size, and position of stomata on varieties of wheat and the relation of these to the entrance of the mycelium of stem rust. He noted (*v. 3, p. 199*) that the waxy bloom of glaucous varieties reduces the stomatal opening to "a crack so narrow that the promycelial threads of rust fail to enter it."

Hursh (6) investigated the number and size of stomata on wheat varieties and found no correlation with rust resistance. He suggested that the degree and duration of the stomatal opening are probably of more importance and greater influence in the entrance of germ tubes than either the number or the size of the stomata.

Allen (2), while studying the early stages of infection of *Puccinia triticina* on Little Club wheat, found that in material fixed in the morning many of the germ tubes had just entered the host and formed the substomatal vesicles. In the material fixed in the afternoon she found that infection was always more advanced and most of the substomatal vesicles had sent out infecting hyphae. She suggested that this daily rhythm might be due to the fact that the time of entrance is conditioned by the daily stomatal movements of the host, and that entrance waits upon natural opening of a stoma rather than upon mechanical force or chemical action.

Allen (1) studied the behavior of three physiologic forms of *Puccinia graminis tritici* on Khapli emmer and found that "the appressoria of all three forms of the fungus secrete some substance, which, if present in sufficient amount, penetrates and kills the guard cells and alters their walls. So far as can be judged by appearances, it is the same substance in all three, but varies in quantity. * * * On comparing the percentage of entries with the degree of stomatal injury, it is to be noted that where injuries are least serious the entries are greatest in number." In these cases the stomata probably closed as soon as the substance penetrated and killed the guard cells, for any outside influence which affects the guard cells and decreases their turgor tends to close the opening between them. The indications are that the stomata must open and remain open for a time, in order that the rust fungus may reach the interior of its host.

EXPERIMENTAL METHODS

The stomatal movements of several wheat varieties were studied in the uniform rust nursery at St. Paul, Minn., to determine, in a rather general way and under natural conditions, the influence of environmental factors on stomatal behavior of different varieties.

⁴ Reference is made by number (*italic*) to "Literature cited," p. 947.

The field observations were supplemented by greenhouse studies in an attempt to control stomatal movements and to ascertain whether germ tubes of the fungus were able to enter susceptible wheat seedlings through closed stomata.

The stomata were observed directly under the low-power (16-mm.) objective of a standard microscope, a method suggested by Lloyd (8). This was to obviate the difficulties encountered when the epidermis is stripped from the plant and plunged into absolute alcohol as a fixative (6, 7, 9). After a little practice it is very easy to draw the plant part to be examined across the microscope stage and to focus on the stomata. Under favorable conditions the movements may be followed very closely for a time. However, in the present work the observations were made as quickly as possible, in order that the stomata might not change as a result of the disturbance of the plant under observation. The individual openings were not measured, but the condition of the majority of the stomata in several microscopic fields on certain plant parts was determined and taken as typical for that particular wheat variety. Four classes were arbitrarily estab-

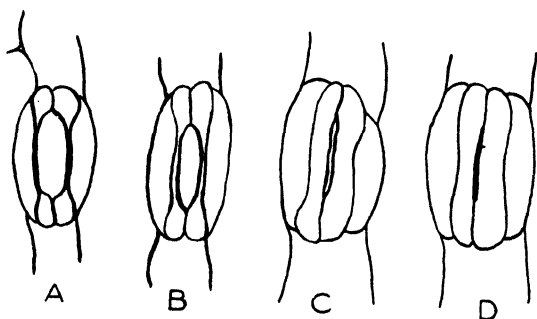


FIGURE 1.—A diagrammatic sketch giving the standards used in the determination of stomatal openings: A, Wide open; B, half open; C, narrow slits; D, closed

lished to express the degree of stomatal opening:

Wide open, approximately half open, narrow slits, and closed. (Fig. 1.)

Of course, the shape and structure of the stomata vary somewhat for the different cereal varieties, but in general the four established classes served the purpose very well.

If the stomata were wide open or half open, the germ tubes of the fungus could enter easily. If

the stomata were closed, the fungus was kept outside the host; and if the openings were narrow slits, it was assumed that the fungus was kept out or else entered with difficulty and by the exertion of mechanical force.

The following wheat varieties were studied in the field: *Triticum vulgare* (Little Club, C. I. 4066; Quality, C. I. 6607; Marquis, C. I. 3641; Baart (Early Baart) C. I. 1697; Haynes Bluestem, C. I. 2874; Reward, C. I. 8182; Ruby, C. I. 6047; Kota, C. I. 5878; Ceres, C. I. 6900; Marquillo, C. I. 6887; Webster, C. I. 3780; Hope, C. I. 8178); *Triticum durum* (Mindum, C. I. 5296; Arnautka, C. I. 1493; Kubanka, C. I. 2094; Acme, C. I. 5284; Velvet Don, C. I. 1445); and *Triticum dicoccum* (Khaphi emmer, C. I. 4013 and Vernal emmer, C. I. 3686).

They were selected from the standard variety rows of the uniform rust nursery at St. Paul. Little Club and Quality were chosen as the two wheats which are extremely susceptible to stem rust whenever they are grown at St. Paul. Marquis, Baart (Early Baart), Haynes Bluestem, Reward, and Ruby usually are heavily rusted at St. Paul unless conditions are particularly unfavorable for the development of

infection. Mindum and Arnautka are very susceptible to most of the rust forms which attack the durumms.

Infection on Kota and Ceres varies greatly from season to season, but even when the percentage of infection is high the type of infection is always the resistant one, with small limited pustules and signs of chlorosis and necrosis in some cases. Kubanka and Vernal emmer are usually resistant in the field also. Marquillo, Webster, Hope, Acme, Velvet Don, and Khapli emmer are always very resistant to stem rust at St. Paul. Very often the last-named varieties are almost free from rust.

The plants in the uniform rust nursery were sprayed repeatedly with spores of a large number of physiologic forms of stem rust, so that the chances for development of rust infection usually are greater there than in other sowings or in other localities.

STOMATAL BEHAVIOR IN THE FIELD

Many preliminary field observations were made under a wide variety of conditions before any definite conclusions could be drawn. The notes were taken during June and early July, from the time the plants were approaching the boot stage until after the heading of most varieties. During this time rust infection usually takes place. The inoculum probably is most abundant during the latter part of this period, but artificial epidemics are produced in the uniform rust nursery by spraying repeatedly with urediniospores of many physiologic forms of stem rust.

Loftfield (9) found a definite daily rhythm in the stomatal movements of cereals. Under favorable conditions the stomata open at sunrise, remain open during the morning, close gradually during the late afternoon, and remain closed all night. If conditions are unfavorable, most of the stomata remain closed during the day or are only partially open for an hour or so after sunrise. Night opening does not occur under ordinary conditions, favorable or unfavorable.

At first different parts of the plants were studied to see if the stomata of all parts behaved similarly. Loftfield (9, p. 76) found that the age and degree of maturity of a plant affected the behavior of the stomata and the readiness with which the stomata functioned. He found also that the degree of succulence of a plant affected the behavior of its stomata. Likewise, the age and the succulence of different plant parts might affect the behavior of stomata of those parts. A leaf blade of wheat is likely to be more succulent than the stem, and the young leaves more succulent than the older ones. On older, more mature plant parts many of the stomata function poorly or remain closed permanently, and the behavior of the stomata in a given area is not at all uniform.

In a particular variety of wheat the stomata of the youngest leaf respond to favorable stimuli more quickly than those of any other plant part. The stomata of the youngest leaf open first after sunrise. In the case of the older leaves the stomata respond to the sunlight a little less quickly perhaps; at any rate the stomatal behavior is less uniform, and most of the stomata lag somewhat in the opening process. Also, the stomata on the sheath of a plant, about midway between the first and the last node, lag a little behind the stomata of the young leaves; by the time the stomata of the young leaves have

opened wide those of the sheath may be only partly open. It may be that the light intensity is slightly lower and accounts for the somewhat slower opening of stomata of the sheath; or it may be merely the age of the sheath as compared with that of the leaf. The stomata on the neck or peduncle lag in opening even more than those on the sheath. They open more slowly and usually less widely than stomata of the young leaf. In this case there should be no decrease in light intensity to account for the slower response. The succulence of the stem tissue may be less than that of the young leaves, the relative humidity of the air about the heads and necks may be less than the

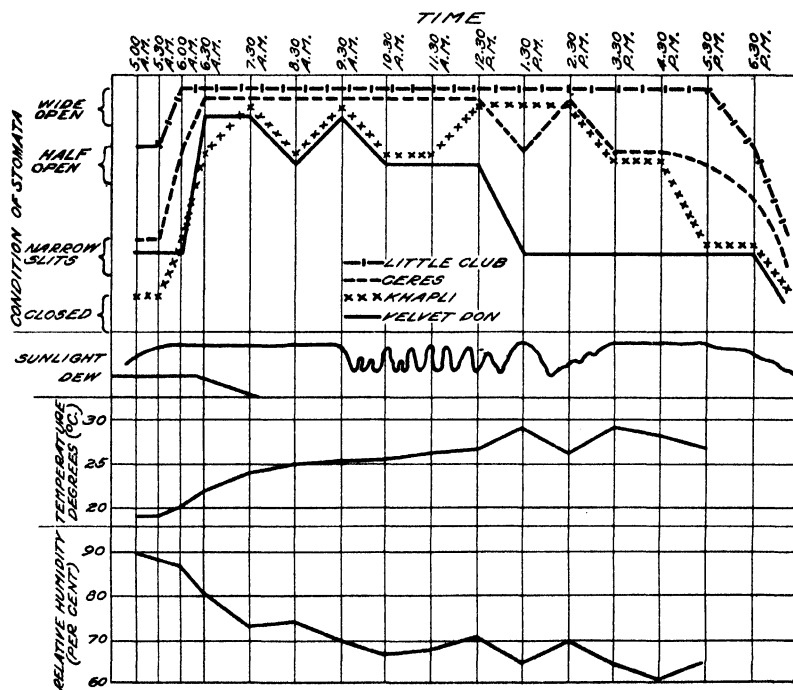


FIGURE 2.—The condition of stomata on four varieties of wheat in the field throughout a typical day during the first half of July at University Farm, St. Paul, Minn. The sunlight, dew, temperature, and relative humidity of the air were recorded at the time the stomata were observed

relative humidity about the lower parts of the plants, or there may be temperature differences in those regions; but it is impossible to say what causes the slight delay in opening of the stomata. The differences in stomatal behavior on different plant parts, although not great, are noticeable, particularly at certain periods of the day when the openings are changing rather rapidly. In all the field observations in which stomatal movements of wheat varieties were studied, care was taken that the comparisons were made between similar plant parts.

All the wheat varieties studied followed, in a general way, the trend of the daily rhythm in stomatal movements which Loftfield (9), observed in all cereals. However, varieties differed greatly in th

details of stomatal behavior. The stomata of some varieties, Little Club, Baart (Early Baart), Quality, and Reward, usually opened almost immediately after sunrise, and under most conditions remained wide open until late in the afternoon. Stomata of these varieties respond very quickly to a definite, favorable stimulus, but they are not very sensitive to adverse changes in light, temperature, humidity, or to an increased transpiration of the plants. In other varieties, Marquis, Haynes Bluestem, Ceres, Ruby, Arnautka, and Mindum, the stomata usually opened a little more slowly in the early morning and were a little more sensitive to external conditions during the day. Under ordinary conditions, however, they remained wide open or partly open most of the day. The stomata of other varieties, Webster, Hope, Acme, and Velvet Don, opened very slowly in the morning and closed again rather early in the day unless conditions were very favorable for stomatal opening. Often, most of the stomata on these varieties remained closed during a large part of the day. Still other varieties, Kota, Marquillo, Khapli, and Kubanka, were intermediate with respect to the behavior of their stomata. Many observations were made under different environmental conditions, but it is impossible to give all the details here. Figure 2 shows the general trend of stomatal movements in four wheat varieties during a typical day early in July. The important point is that there are variations in stomatal behavior under the same environmental conditions, according to the variety of wheat observed.

With respect to the problem of rust infection, the behavior of stomata during the early morning hours immediately after sunrise is of the greatest importance. At that time the plants are covered with dew, so that, provided the temperature is not too low, conditions are ideal for the germination of rust spores lodged on the plants. If the stomata open early, a large proportion of the germinating spores effect entry before the dew is gone from the plants, and, once inside its host, the fungus develops according to its compatibility with the protoplasm of that particular host. The extent of its mycelial development and the formation of fruiting pustules depend to a large extent on the structure of the host (6), but the early stages of infection probably are almost entirely dependent upon the physiology of host and parasite.

A few selected wheat varieties were studied in the field so that some data might be obtained on stomatal movements during the critical hours of the early morning. The results of one series of observations are given in Figure 3. Six varieties of *Triticum vulgare* were chosen: Little Club and Quality, because they are extremely susceptible to most forms of stem rust; Marquis, because it usually is susceptible to a large number of rust forms; Kota, because its infection varies greatly under field conditions and it seems to be intermediate in its field resistance to stem rust; and Hope and Webster, because they usually are highly resistant to rust in the field, Hope being virtually free from infection in most cases. The first observations were made at 5.10 a. m., 20 minutes after sunrise. The stomata of Little Club and Quality were already partly open; those of Marquis appeared as narrow slits; and those of Hope, Webster, and Kota were still closed. The graph indicates the rapidity with which the stomata of each variety opened. A heavy dew was on the plants until 6.20 a. m., and then it gradually disappeared, so that at 8.20 a. m. the plants were

dry. If it is assumed that there was sufficient dew to protect the fungus germ tubes until about 7.30 a. m., it is found that there was approximately a 1-hour period during which the fungus might enter Hope and Webster plants, approximately $1\frac{1}{2}$ hours during which Kota could be entered, 2 hours during which Marquis could be entered, and probably $2\frac{1}{2}$ hours during which the fungus might enter Little Club and Quality. If the end of the heavy dew is taken as the limit for the survival of the germ tubes, then the periods of possible entry are considerably shortened; $1\frac{1}{2}$ hours for the entry of Little Club and Quality, less than 1 hour for Marquis, about one-third of an hour for Kota, and no time for Webster and Hope because their stomata were not sufficiently open until the heavy dew had disappeared. At any rate, it is apparent that some wheat varieties are in a receptive

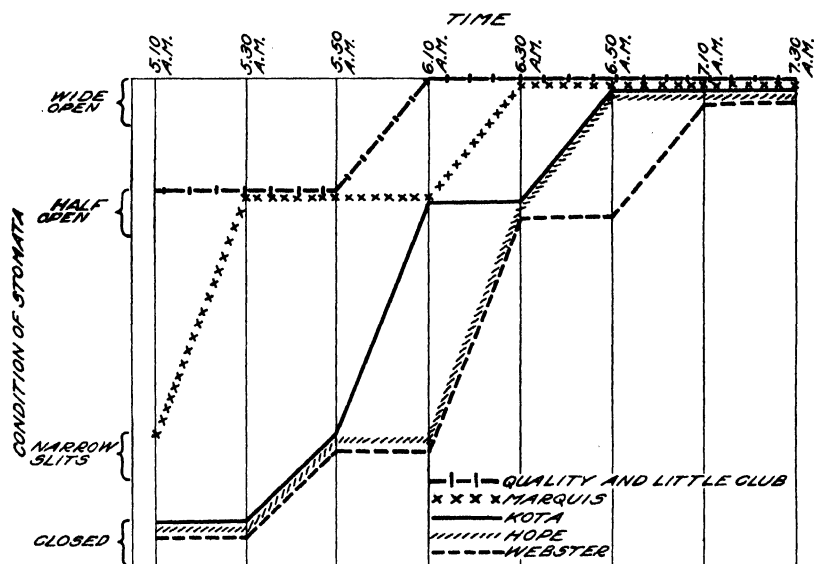


FIGURE 3.—The condition of stomata on wheat plants in the field during early morning hours. Observations started 20 minutes after sunrise on July 3, 1928, at University Farm, St. Paul, Minn.

state for longer periods than others. If similar conditions prevail throughout the growing period, it is easy to see that a large proportion of germinating rust spores will gain entrance to some varieties and may prove to be very effective inoculum, while on other varieties the great proportion of germinating rust spores will be excluded and only a relatively few germ tubes will enter and infect the host.

STOMATAL BEHAVIOR IN THE GREENHOUSE

Stomatal movements in Marquis wheat were studied in the greenhouse at University Farm during January, 1926. Later experiments included several other wheat varieties: Little Club, Kota, Webster, Hope, Ceres, and Marquillo of the *Triticum vulgare* group, and Khapli emmer. The same daily rhythm was noted in 1-week-old seedlings and in the older plants. Light seemed to be the most

important factor influencing the degree of opening, for during the winter months the stomata began to open between 7 and 8 a. m. and started to close between 3 and 4 p. m. Many attempts were made to prolong the period of opening, but very few were successful. Very young seedlings and older plants seemed to have established a daily rhythm in stomatal movement, which was scarcely to be altered by artificial light, humidity, or temperature.

THE EFFECT OF LIGHT

As the general trend of the stomatal movements in cereals seems closely correlated with sunlight, it was advisable to find out how quickly the stomata would respond to light in the greenhouse. For this purpose Little Club, Ceres, Marquillo, and Hope were grown in the greenhouse until they approached the boot stage. The plants were succulent and vigorous, and the stomatal movements followed the definite normal rhythm expected under favorable conditions. One series of observations was made during May, when the sun rose between 5.30 and 5.45 a. m. At 6 a. m. the sun was shining in one of the houses, while the other house was still shaded. The temperature in both houses was 20° C. One lot of plants was transferred from the shaded to the lighter house and placed directly in the rays of the sun. Although the sun was still low on the horizon and the slanting rays were not intense, there was a very decided response in the plants. Within 5 minutes after the transfer to direct sunlight, the stomata of leaves of Little Club started to open and most of them appeared as narrow slits. Within another 4 minutes most of the stomata of these leaves were half open; and after 20 minutes in direct sunlight nearly all were wide open. Ceres also responded quickly, though less so than Little Club. Within 10 minutes after the transfer some of the stomata on leaves of Ceres appeared as narrow slits, although many remained closed. Twenty minutes after the transfer some of the stomata of Ceres were half open, many appeared as narrow slits, and many others were still closed. One hour after the transfer most of the stomata of Ceres were partly open, but very few were wide open until later in the morning. Marquillo and Hope responded very slowly as compared with Little Club and Ceres, and it was easily noticeable that the stomata on very young leaves reacted more quickly than those on the older leaves. Twenty minutes after the transfer a few stomata on the leaves of Marquillo and Hope appeared as narrow slits, but by far the majority of stomata were closed. Even after one hour in direct sunlight many of the stomata on Marquillo and Hope were closed, some were in the narrow-slit stage, and only a very few were half open.

Parallel observations were made on the stomata of the plants left in the shaded house. At 7 o'clock, one hour after the first lot of plants had been transferred to direct sunlight, the stomata on leaves of Ceres and Little Club in the shaded house were just beginning to open and most of them appeared as narrow slits. The stomata on leaves of Marquillo and Hope in the shaded house were still tightly closed. The direct sunlight did not reach this house until about 9 o'clock.

It is evident that light is a very potent factor in stomatal behavior, and that stomata of some varieties of wheat may respond to the

stimulus of sunlight more rapidly than those of other varieties. The other conditions of the experiment were approximately the same. All the plants were at the same stage of development and all were vigorous and succulent. The temperature in the two greenhouses was the same (20°C.) at the beginning of the experiment, but there was a very gradual rise of temperature in the sunny house so that at 7 o'clock it was 23° . The relative humidity of the two houses varied from 70 to 75 per cent. Sunlight was the important variable in this experiment, and seems to account for the earlier opening of stomata on plants kept in the lighter house.

During the winter months a few experiments were made with artificial light. One-week-old seedlings of Little Club and Marquis wheats, which had been growing on the greenhouse bench and had established a normal daily rhythm in stomatal movements, were transferred to artificial-light rooms and placed about 2 feet below a 200-watt lamp. The transfer was made late in the afternoon, and when the plants on the greenhouse bench were examined just before the transfer the stomata were closing. It was hoped that the artificial light would cause the stomata to open again, but it did not. The plants were examined at intervals during the evening, but the stomata were closed. Check plants in the greenhouse were examined at the same intervals and their stomata were closed. During the period of observation the temperature of the light room was about 22°C. , and the relative humidity was about 50 to 60 per cent; the temperature of the greenhouse varied from 16° to 19° , and the relative humidity was about 50 to 60 per cent. Repetitions of the experiment with seedlings, and also with older plants, gave the same results. Artificial light did not cause the stomata to open during the night after they had once closed.

In another experiment the conditions were varied slightly; the plants were transferred early in the afternoon of a bright sunshiny day, so that the stomata of the leaves were wide open at the time the plants were first exposed to artificial light. At 2.15 p. m. the temperature in the greenhouse was 25°C. and the relative humidity 60 per cent. The plants had been exposed to bright sunlight all day, and the stomata were wide open. One lot of plants was transferred to the light room as in the previous experiments, and another lot was left on the greenhouse bench. In the light room the temperature was 22°C. and the relative humidity 50 to 60 per cent. The first observations were made one hour after the transfer. In the light room most of the stomata were half open, but none were wide open. In the greenhouse the plants were shaded and the temperature had dropped to 22° . Most of the stomatal openings were merely narrow slits, although a few were half open. Observations were made hourly until 11 p. m. During that period the stomata of plants in the light room followed the normal rhythm, so that by 11 o'clock most of the stomata were closed and only a very few remained half open. The stomata on the plants in the greenhouse were all closed soon after sundown. Either the artificial light was not strong enough or the daily rhythm had become so well established that it could not be altered to any extent. Although some plants were kept in the light room for several days, their stomata did not remain open during the night.

THE EFFECT OF MOISTURE

Moisture and the relative humidity of the atmosphere have considerable influence on the stomatal movements of most green plants. Loftfield (9, p. 75) stated that a high atmospheric humidity permits stomata to open wider and remain open longer than a low humidity, and that when the leaves of a plant are wet by dew or rain or wet artificially the stomata usually open if closed, or open more widely if partially open.

Potted wheat plants were subjected to saturated atmospheres and to artificial wetting to determine whether the excessive moisture would prolong the period of stomatal opening after sunset. In the first experiment, made during the winter, 10-day-old seedlings of the varieties Little Club and Webster were placed under bell jars on the greenhouse bench. At 6 p. m., an hour or more after sunset, when the stomata were closed, one lot of seedlings was sprayed with water until the plants were thoroughly wet. A second lot was subjected to a saturated atmosphere under a bell jar without a direct spraying of the plants, and the third lot was left in the relatively dry air under a bell jar. The temperature in the greenhouse was about 12° C. throughout the evening. Stomata were observed again at 9 o'clock to see whether the excessive moisture had induced opening. All stomata in the three lots of plants were closed, so that moisture seemed to be ineffective in causing stomata to open after they had once closed. Similar experiments with plants approaching the boot stage of development gave similar results.

A second experiment was made to determine whether excessive moisture would cause open stomata to stay open longer than those of plants kept in relatively dry air. The experiment was started at 2 p. m. one clear January day. Khapli, Marquis, Kota, and Webster plants, all in the 4-leaf stage of development, had been on the greenhouse bench in a clear diffuse light. The stomata of the first three varieties were open, but those of Webster were gradually closing and were in the narrow-slit stage. The temperature was 23° C. at 2 p. m. The plants of one lot were sprayed until the leaves were thoroughly wet and were then placed in a saturated atmosphere under bell jars. Check plants were left on the greenhouse bench. The stomata were examined at 4.30 p. m., when the light was decreasing rapidly and the short winter day was drawing to a close. At that time the temperature in the greenhouse had dropped to 19°. All the stomata of the check plants and all those of the wetted plants in the saturated atmosphere under the bell jars were closed. The excessive moisture had not kept the stomata open.

One more experiment was made with very young wheat plants. Seeds of Mindum wheat were germinated in moist chambers, and before the green leaves appeared each seedling was placed in an individual moist chamber, so that the plants were exposed only to a saturated atmosphere from the beginning of their development. The very young green leaves were kept constantly wet and most of the stomata remained open or partly open after sunset and during the night. Observations were made at intervals, and conditions remained the same for 60 hours. At the end of that time the seedlings were removed, for they did not thrive under the close and excessively wet conditions of the moist chamber. Soon after the excess moisture evaporated from the leaf surfaces the stomata closed, and from then

on light seemed to be the most important factor influencing stomatal movements.

THE EFFECT OF TEMPERATURE AND MOISTURE

In many of the experiments to determine the effect of moisture on stomatal behavior, the temperature varied so much that it was decided to ascertain whether it modified the effect of moisture. There is a possibility that, if the temperature is favorable for stomatal opening, excessive moisture might cause the stomata to remain open at night.

The following experiment was made: Little Club and Webster wheats, in the 4-leaf stage, were kept at temperatures of 12°, 20°, and 27° C. during the day, and the stomatal movements were observed closely. The light was about the same in the three locations, and there was a normal rhythm in stomatal movements at all three temperatures. In the middle of the afternoon one lot of plants at each temperature was sprayed and placed in a saturated atmosphere under bell jars. Frequent examinations were made during the afternoon and evening, but all the stomata closed soon after sunset. Neither temperature nor moisture altered the normal behavior to any extent, for the stomata closed when the light stimulus was removed.

Inasmuch as the stomata of the cereals are closed during the night and sometimes for considerable periods during the day, it is doubtful whether the rust fungus is able to effect entry at all times.

ENTRANCE OF THE PARASITE THROUGH CLOSED STOMATA

It is essential to know whether the germ tubes of the stem-rust fungus normally enter a host plant through closed stomata. If they do so normally and easily, then stomatal behavior should have no effect on the amount of infection under conditions favorable for the development of rust.

This phase of the problem was studied in the greenhouse with seedling plants and with certain physiologic forms of stem rust to which most of the varieties of wheat used were very susceptible. Seedlings were used because the behavior of their stomata is more uniform over relatively large plant surfaces than that of stomata on older plants. Dust and other foreign particles often lodge between the guard cells on the older plants and keep the stomata open. Seedlings can be protected from such accidents and, moreover, can be handled more easily and quickly. The probability of successful infection when inoculations are made in the greenhouse seems to be greater with seedlings than with older plants. Inoculated seedlings are usually kept in the moist chamber for 48 hours after inoculation, but it is necessary that older plants be kept there for 72 hours or longer. Then again, the first pustules appear on seedlings within 7 to 10 days after inoculation, but with older plants the incubation period usually is longer. Thus, in an experiment of this kind, quicker and more reliable results should be obtained with seedlings than with older plants.

The first experiments were made with 3-day-old seedlings of Webster, Kota, and Little Club. The seeds were germinated in a moist chamber which protected them from dust and other foreign material. At the end of the third day, just before inoculation, the stomata were

examined, and as nearly as could be determined all were closed. The seedlings were atomized and inoculated with urediniospores of *Puccinia graminis tritici* form 17. They were then placed in moist chambers, which were kept in darkness so that the stomata would remain closed. Both Little Club and Kota are susceptible to form 17, giving 4 and 3+ reactions, respectively (14). Webster is moderately resistant to this form, the reaction varying from 2- to 3- (15). A check lot of seedlings was atomized and placed in another moist chamber for observation at intervals to see if the stomata remained closed. Spore-germination tests were made at the same time in order to be sure that effective inoculum was present. The inoculated seedlings were left in the dark moist chamber for 24 hours and then removed to open culture dishes, where they were supplied with Shive's solution (12) daily. Nine days after inoculation, single, minute uredinia appeared on the tips of two out of four Little Club seedlings and on one out of four Webster seedlings. The uredinia were so small as to indicate that a single germ tube had entered in each case. In the case of Little Club, the pustules were at the tips of the seedlings, and it was concluded that the fungus had entered through the open hydathodes at the tips of the leaves. In the case of Webster this was not true, for the pustule was located some distance behind the tip. The fungus either forced its way through a closed stoma or a few of the stomata were open during the time the plants were in the moist chamber.

In the next experiment greater precaution was taken to restrict the inoculation court. A second series of 4-day-old seedlings of Little Club, Kota, and Webster was inoculated with *Puccinia graminis tritici* form 17. The spores were carefully placed about one-half inch back of the leaf tip after the plants had been examined and the stomata found closed. One lot of each variety was placed in a moist chamber in the dark and left for 24 hours. Another lot was placed in moist chambers and left on a table where the light would reach them after 10 to 12 hours. At the end of 24 hours the plants were transferred to Shive's solution. Nine days after inoculation, two or three pustules appeared about 1 inch from the tips of each of the Kota seedlings which had been exposed to light 12 hours after inoculation. Infection seemed to be similar to that normally obtained in the greenhouse, except that the pustules were fewer. The Webster and Little Club plants of that lot did not thrive under the conditions of the experiment, and no pustules appeared during the first 12 days after inoculation. None of the plants kept in the dark moist chamber became infected during the 12 days.

Some time later, 24 Mindum seedlings were inoculated with *Puccinia graminis tritici* form 21, which produces a type 4- infection on this variety (14). The inoculum was placed about three-fourths of an inch from the tips of the seedlings to preclude the entry through hydathodes. The inoculated seedlings were atomized and placed in a moist chamber for six hours, during which time the stomata remained closed. In germination tests 80 per cent of the spores had germinated during the six hours and had produced germ tubes which would be long and vigorous enough to enter the host, unless they were prevented by the closed stomata. After six hours the seedlings were removed from the moist chambers, placed in culture jars, and supplied with

Shive's solution. Care was taken to prevent a collection of excess moisture on the leaves, in order that spores left on the surfaces might not germinate later and effect entry when the stomata were open. The seedlings grew well in the nutrient solution, and no signs of infection appeared during the first eight days following inoculation. On the ninth day very minute pustules appeared on 2 of the 24 seedlings, about three-fourths of an inch from the tips. The pustules were so very small that on first examination they seemed to be merely remains of the inoculum. Their development was very slow, and it was obvious that each pustule had originated from a single infecting germ tube. The inoculated plants were examined twice daily for 15 days. At the end of that time 20 seedlings were healthy and free from infection, 2 were free from infection but the tips of the leaves were shriveling, and 2 each had single minute rust pustules three-fourths of an inch from the leaf tip. The closed stomata apparently had excluded most of the germ tubes. A 6-hour period in the moist chamber seems a short time to permit infection, but it has been found that as short a period as three hours is sufficient for a considerable number of entries when the stomata are open.

The results of still another test are given in Table 1. While the numbers of seedlings used in this trial were very small, the results are very definite and indicative of the results obtained when larger numbers were used. On the seedlings inoculated at the leaf tips, where the hydathodes served as avenues of entrance, pustules appeared in from 5 to 7 days after inoculation, regardless of whether the seedlings had been in dark moist chambers or in those exposed to the light for a part of the time. On the seedlings inoculated three-fourths of an inch back of the tip, pustules appeared from 7 to 10 days after inoculation, but only on those which had been in moist chambers exposed to the light. No pustules appeared on seedlings kept in the dark moist chambers, probably because the stomata were closed while the rust spores were germinating and the germ tubes were in condition to enter the plant.

Sections were made of a few of the inoculated seedlings to see whether there was any evidence that the germ tubes had forced their way between the guard cells. Many of the germ tubes had formed appressoria and on some of them there were short projections which seemed to fit into the depression between the two guard cells although the stoma was closed. However, there was only one case which possibly might be interpreted as a forced entry. An appressorium was formed over a stoma, and in cross section a thick, wedge-shaped projection appeared to have forced the guard cells apart until the fungus had penetrated about half the depth of the stomatal slit. The inner half of the stoma was closed, and the walls of the guard cells were pressed tightly against each other. The fungus may have wedged its way into the stoma, or the stoma may have been partly open at the time the fungus reached it and may have closed again before the fungus could enter. The conclusions drawn from the prepared slides were that the rust fungus generally does not enter the host plant if the stomata are closed.

TABLE 1.—*The results of inoculating Little Club and Webster wheat seedlings with Puccinia graminis tritici form 17 at and near the leaf tips when the stomata were closed, and incubating them for 24 hours in moist chambers kept in darkness or exposed to light after 12 hours*

[The numerator of the fraction indicates the number of infected seedlings; the denominator, the total number of seedlings]

Variety used	Date of inoculation (1927)	Inoculation court	Moist chamber conditions	Infection at specified times after inoculation			
				5 days	7 days	8 days	10 days
Little Club	Dec. 5	Tip of seedling	Dark	$\frac{2}{3}$	$\frac{3}{3}$		
			Light	$\frac{3}{3}$	$\frac{3}{3}$		
Do.	Dec. 7	$\frac{3}{4}$ inch from tip	Dark	$\frac{0}{5}$	$\frac{0}{5}$	$\frac{0}{5}$	$\frac{0}{5}$
			Light	$\frac{0}{5}$	$\frac{1}{5}$	$\frac{4}{5}$	$\frac{4}{5}$
Webster	Dec. 5	Tip of seedling	Dark	$\frac{0}{2}$	$\frac{2}{2}$		
			Light	$\frac{0}{2}$	$\frac{2}{2}$		
Do.	Dec. 7	$\frac{3}{4}$ inch from tip	Dark	$\frac{0}{5}$	$\frac{0}{5}$	$\frac{0}{5}$	$\frac{0}{5}$
			Light	$\frac{1}{5}$	$\frac{2}{5}$	$\frac{3}{5}$	$\frac{3}{5}$

DISCUSSION

In the study of disease resistance, and especially the rust resistance of cereals, it has been impossible to find a universal explanation for the resistance of some varieties and the susceptibility of others. There apparently are several types of resistance to stem rust. Many investigators have studied the so-called physiologic resistance of certain cereals and the phenomena associated with infection of a resistant host by a rust fungus (4, 10, 13, 16), and Hursh (6) has definitely shown that the morphology of the host may have a pronounced effect on the development of rust in certain varieties of wheat. The present writer has shown that there may be a third factor termed "functional resistance," which may enable some varieties to escape infection because of their characteristic stomatal behavior.

As the stomata of the cereals constitute the avenues of entrance for the germ tubes of rusts, and stem-rust germ tubes do not enter the host unless its stomata are open, the factors that influence the stomatal movements would indirectly influence the course of rust infection. The stomata of cereals are always closed at night, and remain so until sunrise or later. Hence, even though many urediniospores on the plant surfaces germinate during the night, few or no germ tubes can enter the host until the return of the sunlight causes the opening of stomata. During the growing period in the upper Mississippi Valley there usually are heavy dews which often remain on the plants for a considerable time after sunrise. Naturally, the dew remains longer on plants in the heavier stands of grain, but even on those in moderately light stands the plants are wet for some time after sunrise. This provides an ideal condition for the germination of rust spores and for the survival of germ tubes which may have been formed during the night. By far the greatest number of infections occur during this critical period between sunrise and the disappearance of the dew. The stomatal behavior of the cereal hosts enters into the problem at this time.

If the stomata of a particular variety open quickly in response to the stimulus of sunlight, it is obvious that the germ tubes may enter sooner and that the period favorable for entry is longer than in the case of another variety whose stomata respond very slowly and do not open for an hour or more after sunrise. After the dew disappears and the surfaces of the plants become dry, most of the delicate fungus germ tubes die, unless they have grown into the plant. If most of the stomata of the plant remain closed until after the dew disappears, most of the germ tubes will perish and never enter the host. Stomatal movements probably vary greatly under different environmental conditions, but it seems highly probable that under ordinary conditions in the upper Mississippi Valley the stomatal behavior of some wheat varieties usually is such as to exclude a large number of the stem-rust germ tubes.

For many years certain varieties of wheat grown at University Farm, St. Paul, Minn., have been consistently resistant to stem rust when grown in the field, in spite of the fact that they are susceptible to some forms of *Puccinia graminis tritici* when inoculated in the seedling stage in the greenhouse. Rust surveys have shown that forms of rust which can attack these varieties are present in the field. Although there should be plenty of inoculum, little or no rust develops on them. Why is it? Is there, as some workers have suggested, a "mature plant resistance" which is not evident in the seedling stages but which develops later in the life of the plant? Or can some of this resistance be explained on the basis of stomatal behavior of the wheat variety in question?

Kota is very often resistant to stem rust in the field at University Farm. The infection varies from a trace, as in 1919, 1921, and 1922, to 12 to 20 per cent, as in 1925 and 1927, or even as high as 33 per cent, as in 1920. Hursh (6) has shown that the structure of Kota limits the size of the rust pustules after the fungus enters the host tissues, but in many seasons Kota is almost free from rust, and there is so little evidence of infection and subsequent pustule formation as to indicate complete exclusion from the host. Likewise, the amount of infection on Kota varies according to locality. In some of the uniform rust nurseries the percentage of infection is relatively high (25 to 35 per cent at Madison, Wis., and 38 to 75 per cent at Morris, Minn., in 1927), while in other nurseries only a small amount of rust develops (1 to 2 per cent at Manhattan, Kans., and 4 to 10 per cent at Fargo, N. Dak., in 1927). Forms of stem rust to which Kota is susceptible may be present in all of the nurseries (forms 18 and 21 of *Puccinia graminis tritici* were isolated from Madison, Morris Manhattan, and Fargo, in 1927), but the percentage of infection varies greatly.⁵ It is probable that stomatal behavior may be partly responsible for some of these differences.

In the field there is great variation in the amount of stem rust on Webster wheat, which is moderately resistant to those forms of *Puccinia graminis tritici* with which it has been inoculated in the greenhouse. In the uniform rust nursery at University Farm, only 5 to 10 per cent of stem rust developed on Webster. On the sandy experimental plots at Coon Creek, Minn., there was 63 to 90 per cent

⁵ Unpublished results of Stakman and his coworkers, made available through the kindness of Dr. E. C. Stakman.

infection on Webster in 1927, although the pustules were all relatively small and the majority of them never ruptured the epidermis. Stomatal behavior was not studied at Coon Creek, but it is certain that a great many more entries were effected there than at University Farm.

Velvet Don has been grown in the field at University Farm since 1909, and during that time severe epidemics of stem rust were produced artificially. During the whole 19-year period, however, Velvet Don has always been very resistant and usually not more than a trace to 5 or 10 per cent of stem rust develops. When seedlings of this variety are inoculated in the greenhouse with *Puccinia graminis tritici* form 21, a type 4 infection results, indicating marked susceptibility to that form. When greenhouse inoculations are made later, on plants in the 4-leaf stage, in the boot, or after heading, excellent infection results, an abundance of rust pustules develop, and there is no indication of resistance. What accounts for the marked resistance in the field? The host grows vigorously and should be in suitable condition for infection; the virulent rust forms are present almost every year; but there is very little infection. Preliminary observations have been made on stomata of Velvet Don, and it may be that stomatal behavior accounts for a large part of the field resistance at University Farm. In the greenhouse the older plants are kept in the moist chamber for three days after inoculation, and in that period there probably is considerable time during which the stomata are open and the germinating spores are entering the plants. In the field the periods of stomatal opening probably do not coincide with the periods favorable for spore germination and growth of germ tubes, so that the fungus seldom gets inside the plants of this variety.

There is evidence also that in some years a great number of stem-rust germ tubes enter Khapli emmer grown in the field at University Farm. Khapli has a marked physiological resistance to all the forms of stem rust so far found in the United States, but it is a fact that, under favorable conditions, *Puccinia graminis tritici* enters such a resistant host as readily as it enters a highly susceptible host. The development of the mycelium is never very extensive in Khapli, and fruiting pustules usually are not formed. A cursory field examination of Khapli reveals no evidence of infection, but if the stem of the plant is drawn between the fingers the surface feels uneven and bumpy, owing to the distension of the epidermis over tissues infected by the rust. A microscopic examination of a cross section of the stem shows that the fungus has entered the host, and its mycelium has developed in the chlorophyllous strands of the wheat to such an extent that the epidermis is stretched and distended without being ruptured. In some years this type of infection is very usual on Khapli; in other years Khapli is almost entirely free from rust, the stems are smooth and even, and apparently the fungus has been excluded from the plants. It seems probable that the stomatal behavior of Khapli is an important factor in the exclusion of the rust in these latter cases.

Goulden, Neatby, and Welsh (5) investigated the "mature plant resistance" in a cross between Marquis and H-44-24. They studied the inheritance of field resistance and concluded that it could be explained on a single-factor basis. Although this particular problem

has not been studied, it is possible that the single factor may be stomatal behavior. The present studies indicate that such a physiological tendency or habit is inherited in the same way as are the factors for morphologic and physiologic characters in general.

Stomatal behavior of the cereals evidently is far more important in the phenomena of rust infection than has been recognized heretofore, and it should be studied in greater detail. Stomatal behavior may be a more effectual means of resistance to stem rust than a fundamental protoplasmic resistance or even a morphological resistance. A variety which excludes the rust by its stomatal behavior would be resistant to all rust forms, not merely to some of them. Therefore, the problem of producing satisfactory rust-resistant varieties may be simpler than it has appeared to be.

SUMMARY

Two kinds of resistance to stem rust have been demonstrated previously: Physiological resistance and morphological resistance. To these may be added a third kind, termed "functional resistance."

Some varieties of wheat are resistant to stem rust in the field because of the behavior of their stomata. Such varieties may be susceptible to stem rust in the seedling stages of their development.

Stomatal movements of wheats follow a definite daily rhythm. The stomata open gradually after sunrise, remain open for varying lengths of time, close gradually during the afternoon, and remain closed all night. The daily rhythm of stomatal movements differs considerably in different varieties of wheat. Stomata of some varieties open very soon after sunrise and usually remain open most of the day. Stomata of other varieties open very slowly and remain open only a short time. There also are varieties with an intermediate type of stomatal behavior.

Stomata of the younger and more succulent plant parts open sooner and remain open longer than stomata on older or less succulent parts. Stomata on the young wheat leaves open sooner than most of the stomata on the older leaves, on the sheath, and on the peduncle of the plant. The differences are not great but are appreciable under ordinary conditions.

The critical period for stem-rust infection is in the early morning immediately after sunrise and while the plants are heavy with dew. The fungus easily enters its host if the stomata are open during most of the critical period, but if they are closed during that time the fungus is excluded. There are great differences in the stomatal behavior of some wheat varieties during this critical period.

Direct sunlight seems to be the most important stimulus for the opening of stomata of cereals.

Artificial light, excess of moisture, and excess of moisture and temperature combined did not prolong the period of openness for stomata of cereals in the greenhouse.

From inoculation and histological studies it appears that the stem-rust germ tubes generally, and probably always, enter the host only when the stomata are open. The fungus does not seem to force its way through closed stomata.

A variety of wheat may appear truly resistant to stem rust if the behavior of its stomata is such that most of the inoculum is excluded and rendered ineffective.

There is a correlation between stomatal behavior and the resistance of certain varieties of wheat to stem rust in the field at University Farm, St. Paul. Stomata of the highly susceptible varieties, Little Club, Baart (Early Baart), Quality, and Reward, open soon after sunrise and remain open most of the day. Stomata of some slightly less susceptible varieties, Marquis, Ruby, Haynes Bluestem, Arnautka and Mindum, open a little more slowly after sunrise, but they too remain open most of the day. In the varieties which are highly resistant in the field, Hope, Webster, Acme, and Velvet Don, the stomata open very slowly and close again relatively early in the day. Varieties which are moderately resistant in the field, Kota and Kibanka, have an intermediate type of stomatal behavior.

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THE MINERAL CONTENT OF THE JUJUBE¹

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INTRODUCTION

Since the jujube or Chinese date (*Zizyphus jujuba* Mill.) promises to become a satisfactory tree for southern cultivation, the chemical composition of its fruit begins to assume importance. Church² has analyzed the pulp of several varieties and has made comparisons between its composition and that of the fig and date. Table 1, giving some data taken from Table 1 of his article, shows the composition of the edible matter in the fruit of S. P. I. No. 30488, a variety used in the present study.

TABLE 1.—*Percentage composition of the edible material of jujubes on a moisture-free basis*

S. P. I. No.	Sugars			Acid as anhydrous citric	Protein N×6.25	Crude fiber	Ash	Undetermined
	Reducing	Sucrose	Total					
30488 ^a	35.50	37.86	73.36	0.80	2.73	3.46	1.90	17.7
30488 ^b	30.73	37.90	68.63	.80	3.41	3.41	1.97	21.7

^a Was fully colored.

^b Partially colored. Both were partly wrinkled and soft.

A search of the literature has revealed no data on the mineral content of the jujube, and it was therefore thought advisable to make a mineral analysis. The fruit was collected in the fall of 1928 from two trees, of the varieties designated as S. P. I. Nos. 17752 and 30488, which have been growing in the experiment station orchard since 1917. The fruit was in approximately the same state as Church's first sample in Table 1, fully colored and quite wrinkled.

EXPERIMENTAL PROCEDURE

The fruit was washed, thoroughly scrubbed with a small brush, and dried in the sun; the pulp was separated from the seeds as completely as possible by stripping and scraping off with a knife, and seeds and pulp were dried separately (1) for several days at 70° C. and (2) overnight at 110°. The dried seeds with adhering pulp fragments were then rubbed gently in a mortar, and the powder which separated was added to the dried pulp. The material was then ground, thoroughly sifted, and placed in open beakers in a large desiccator, where it stood for some time before analytical work began. Material intended for

¹ Received for publication May 11, 1929; issued December, 1929. Published with the permission of the director of the experiment station.

² THOMAS, C. C. THE CHINESE JUJUBE. U. S. Dept. Agr. Bul. 1215, 31 p., illus. 1924. (With a chapter on Composition of the Chinese Jujube, by C. G. Church, p. 24-29.)

iron analyses was ground in a porcelain mortar; for the rest a Wiley mill was used. The dried material was kept in the desiccator between times when samples were being taken. Occasionally total-ash determinations were made in order to detect any change in composition.

The analytical methods used were among those recommended by the Association of Official Agricultural Chemists³ except that the potassium was weighed as the perchlorate, and an adaptation of Kennedy's⁴ method was used for iron.

Iron determinations were carried out as follows: 5 gm. of dried material was ashed at a dull-red heat to a gray ash, treated with concentrated HNO_3 , and reashed; the process being repeated, if necessary, till no carbon particles remained. The residue was digested with 2 c. c. of concentrated HCl and 3 drops of concentrated HNO_3 . The solution and the insoluble silica were washed into a 50 c. c. flask and diluted to the mark. In the case of the raisins and dates, which were analyzed along with the jujubes for purposes of comparison, 4 c. c. of concentrated HCl was used and the sample diluted to 100 c. c. Ten cubic centimeter portions were pipetted into glass-stoppered bottles, treated with 10 c. c. of amyl alcohol and 5 c. c. of 20 per cent KCNS solution, the mixture shaken gently, and the alcohol layer pipetted into a colorimeter cup, and the color compared with that of a standard. A standard was made up with analytical iron wire dissolved in H_2SO_4 and oxidized with a little HNO_3 . From the first standard, others of convenient concentrations were prepared as follows: A measured volume of the iron solution was placed in a graduated flask, a measured quantity of 1:4 HCl was added, and the flask filled to the mark with distilled water. Ten cubic centimeter portions of the iron solutions of the fruits had been titrated with 0.2 N NaOH ; and the amount of 1:4 HCl added in preparing the standards was the calculated amount required to make the acidity, volume for volume, of the standards equal to the average acidity of the solutions under examination.

The presence of interfering substances, phosphates and the like, was tested for by making equal mixtures of the standard and the solutions under examination and comparing these with the standard in the colorimeter. The observed iron content of such mixtures in every case compared closely enough with the calculated value to indicate that interfering compounds could not be present in significant amounts.

The raisins and dates used were of standard brands, bought on the market.

³ ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C., 1925.

⁴ KENNEDY, R. P. THE QUANTITATIVE DETERMINATION OF IRON IN TISSUES. *Jour. Biol. Chem.* 385-391, illus. 1927.

ANALYTICAL RESULTS

The results of the analyses are shown in Tables 2 and 3.

TABLE 2.—Percentage of ash and individual mineral constituents in jujubes as compared with dates and seeded raisins

Fruit	Ash ^a in edible material, original basis	Ash in edible material, moisture-free basis	Mineral constituents on a moisture-free basis									
			CaO	MgO	K ₂ O	Na ₂ O	P ₂ O ₅	MnO	Fe ₂ O ₃	SO ₃	Cl	SiO ₂ soluble
Date	1.46	2.04	0.138	0.138	0.964	0.079	0.016	—	0.0096	0.149	0.412	0.122
Seeded raisin	2.04	2.94	.078	.088	1.445	.122	0.031	—	.0169	.118	.040	.171
Jujube S. P. I. No. 17752	1.03	1.84	.100	.083	1.056	.031	.019	0.021	.0021	.034	.076	.005
Jujube S. P. I. No. 30488	1.42	2.36	.132	.083	1.318	.033	.015	.023	.0027	.033	.086	.006

^a For this determination, raisins and dates were used as they came from the package, jujubes after being cleaned and dried for several days in the sun.

TABLE 3.—Composition of ash of jujubes compared with that of dates and seeded raisins

Fruit	Total ash on a moisture-free basis	Percentage composition of ash									
		CaO	MgO	K ₂ O	Na ₂ O	P ₂ O ₅	MnO	Fe ₂ O ₃	SO ₃	Cl	SiO ₂ (soluble)
	<i>Per cent</i>										
Date	2.04	6.63	6.52	45.04	2.36	0.78	—	0.471	7.35	19.06	5.77
Seeded raisin	2.94	2.64	2.97	48.72	4.11	1.06	—	.576	4.01	1.31	4.81
Jujube S. P. I. No. 17752	1.84	5.42	4.46	56.40	1.65	1.04	1.11	.115	1.85	4.08	.28
Jujube S. P. I. No. 30488	2.36	5.60	3.53	54.78	1.29	.64	.99	.115	1.40	3.49	.27

Considering those minerals which are of chief nutritional significance, the two varieties of the jujube studied are seen to compare favorably with the date and raisin as sources of phosphorus and calcium but to be markedly inferior in iron content.

TRANSGRESSIVE SEGREGATION FOR SUSCEPTIBILITY TO SMUT IN AN OAT CROSS¹

By R. J. GARBER, N. J. GIDDINGS, and M. M. HOOVER, *West Virginia Agricultural Experiment Station*

INTRODUCTION

In a recent paper² evidence of transgressive segregation for susceptibility to smut in the oat cross Gopher×Black Mesdag was presented. During the season of 1928 further data relative to this case were collected, and it is the purpose of the present paper to present some of these.

The pertinent literature was reviewed, and a description of the general methods followed in this investigation was given in the article cited above. During the season of 1928 the F₃ families were grown in triplicate plots, but the F₄ and F₅ families were grown in duplicate plots. In all, there were planted 120 seeds of each F₃ family, 80 seeds of each F₄ and F₅ family, and 40 seeds in each parental plot. The parents were interspersed at intervals of 10 plots (20 rows) among the progeny. Each plot in the nursery consisted of two rows 5 feet long. Consequently, with but 20 seeds planted per row, there was ample space for individual plant development.

All seed was treated with smut³ spores about one month before the date of planting (June 16 and 18). A fairly successful smut epidemic was obtained, although in certain places in the nursery there seemed to be a somewhat lower infection than in others, as indicated by the degree of infection of the Gopher parent. Smut notes were taken after all the oats were completely headed and again at harvest time.

F₃ FAMILIES

The percentage of smutted plants and the color of seed of the F₃ families and of the parent stocks grown in 1928 as well as those already reported⁴ are shown in Table 1. Of the 50 F₃ families grown in 1928, 13 showed no evidence of smut infection and 6 gave rather striking evidence of a susceptibility to smut greater than that of the Gopher parent. The other 31 F₃ families showed a range in percentage of smut infection which fell within that of the susceptible parent. It is apparent that the data collected in 1928 are similar to those obtained in 1926. In the latter year each F₃ family was grown in a single plot of three rows only, which accounts in part at least for the somewhat greater variability obtained. Each variate recorded in the frequency distributions for 1926 represents a single plot, whereas each variate recorded for 1928 represents triplicate

¹ Received for publication Feb. 19, 1929; issued December, 1929. Approved as Scientific Paper No. 72 by the director, West Virginia Agricultural Experiment Station.

² GARBER, R. J., GIDDINGS, N. J., and HOOVER, M. M. BREEDING FOR DISEASE RESISTANCE WITH PARTICULAR REFERENCE TO THE SMUT OF OATS. *Sci. Agr.* 9: 103-115. 1928.

³ No effort was made to distinguish between loose smut (*Ustilago avenae*) and covered smut (*U. levis*), although most of the oat smut which occurs naturally in the vicinity of Morgantown, W. Va., is *U. avenae*.

⁴ GARBER, R. J., GIDDINGS, N. J., and HOOVER, M. M. *Op. cit.*

plots. On the basis of progeny tests it was found that 2 F_3 families recorded as "zero infection" in 1926 were wrongly classified. The correction has been made in Table 1.

Of the 150 F_3 families which were grown, 32 showed no evidence of smut infection and 13 showed transgressive segregation for smut susceptibility. Assuming that a single main factor difference is responsible for determining the high degree of resistance, if not immunity, of the Black Mesdag parent, one would expect one-fourth, or 37.5, of the F_3 families to be similar to it in smut reaction, whereas 32 actually were obtained. The difference (5.5 ± 3.6) is not significant.

TABLE 1.—Number of smutted plants in various percentage classes and color of seed among the parents and F_3 families of an oat cross, Gopher \times Black Mesdag, and the reciprocal, grown in 1926 and in 1928, at Morgantown, W. Va.

Parent or progeny	Year grown	Color of seed	Number of plants in smut-percentage classes																Total
			0	2.5	7.5	12.5	17.5	22.5	27.5	32.5	37.5	42.5	47.5	52.5	57.5	62.5	67.5		
Gopher	1926	White	1	1	1	1			3	2	1	1						11	
Black Mesdag	1926	Black	11															11	
F ₃ families	1926	do	7	6	5	3			1	1			1				1	26	
Do	1926	Segregating.	7	8	19	9				2	2	1				3		51	
Do	1926	White	5	6	1	4			2	1	1	2	1					23	
Gopher	1928	do		2	2	1												5	
Black Mesdag	1928	Black	5															5	
F ₃ families	1928	do	4	5	2	1			1									13	
Do	1928	Segregating.	7	6	7	1			2	2	1							26	
Do	1928	White	2	2	6	1												11	

The F_3 families which showed a susceptibility to smut greater than that of the Gopher parent indicate that at least one additional hereditary factor is conditioning smut reaction in this cross. The Gopher parent has consistently proved to be of moderate susceptibility as compared with the highly susceptible progeny lines.

LINKAGE

Some evidence of a genetic linkage between the black-color gene and at least one factor which is causing increased smut susceptibility has been published.⁵ The data obtained in 1928 corroborate those obtained earlier. The six F_3 families which showed transgressive segregation for smut susceptibility were either black seeded or segregated for black-seed color. In 1926 there were also six F_3 families which showed susceptibility to smut clearly beyond the range of the Gopher parent and two additional families were doubtful but showed percentages of infection a little greater than that of the most susceptible plot of Gopher. One of these F_3 families had white seed and the other black.

F_4 FAMILIES

The F_4 families grown in 1927, and already reported, are shown in Table 2. Family 17-10-30 did not show any smut infection in F_3 , but one of the five F_4 families grown from it had a single smutted plant. Further tests in F_5 indicated that this family was truly a

zero-infection strain; hence a correction has been made in the data originally published.

All of the families except one noted as zero infection in F_3 apparently bred true for this character in F_4 . One F_3 family, 16-15-59, produced four F_4 families which showed smut to one which did not; consequently the F_3 should probably have been classed as a low-infection rather than as a zero-infection strain.

TABLE 2.—Percentage of smutted plants among the F_3 families, number of smutted plants in various percentage classes among the respective F_4 families, and the parents grown among the latter, of an oat cross, *Gopher* × *Black Mesdag*, and the reciprocal, grown in 1927.

Parent or progeny	F_3 families		Number of plants in various smut-percentage classes among F_4 families										Total ^a
	Plants	Smutted	0	2.5	7.5	12.5	17.5	22.5	27.5	32.5	37.5	42.5	
	Number	Per cent											
<i>Gopher</i>				3	7	5		1					16
<i>Black Mesdag</i>			16										16
16-15-4.....	64	0	5										5
16-15-8.....	66	1.5	3	1		1							5
16-15-7.....	64	12.5	1	3	1								5
16-15-39.....	60	55.0				2	2		1				5
16-15-16.....	70	0	5										5
16-15-31.....	66	1.5	2	3									5
16-15-1.....	51	13.7	2	1	2								5
16-15-21.....	70	27.1			3			1	1				5
16-15-17.....	70	0	8										8
16-15-35.....	63	0	5										5
16-15-36.....	54	20.4	2	1	1	1							5
16-15-53.....	69	0	5										5
16-15-55.....	70	27.1		1	1	1	1		1				5
16-15-59.....	65	0	1	3	1								5
16-15-64.....	60	0	5										5
16-15-68.....	73	28.8		2		3							5
17-10-1.....	48	0	5										5
17-10-21.....	65	56.9				2	1	1	1				5
17-10-22.....	65	1.5	1	2	1	1							5
17-10-35.....	65	27.7	1		1	2	1						5
17-10-30.....	61	0	5										5
17-10-54.....	63	11.1	3	1		1							5
17-10-62.....	67	0	5										5
17-10-64.....	67	50.7				2	1	1	1				5
17-10-52.....	70	0	5										5
17-10-68.....	55	65.5							1	2	1	1	5
17-10-53.....	68	0	5										5
17-10-75.....	71	57.7				1	2		2				5
17-10-72.....	65	1.5	2	1	2								5
17-10-48.....	65	0	10										10

^a Most of the duplicated parental and F_4 plots contained from 65 to 70 plants. Thirty-two plots of each parent were grown, thus making 16 duplicates comparable to the F_4 families.

It is obvious from Table 2 that all the F_3 families classified as low-infection strains were heterozygous, as they produced both smutted and smut-free F_4 families. On the other hand, all the families which showed a high, and some of those which showed an intermediate degree, of smut infection in F_3 produced in F_4 only families which contained smutted plants.

In Table 3 are shown the F_4 families and the parents among them grown in 1928. The F_3 lines from which these F_4 families were derived were grown in the same year (1926) as the F_3 lines reported in Table 2.

There were eight F_3 lines noted as zero infection which were grown in F_4 . All but one proved to be homozygous for this character. Strain 17-10-15 produced both smutted and smut-free F_4 families,

and hence probably should have been classed as a low-infection rather than as a zero-infection strain.

TABLE 3.—Percentage of smutted plants among the F_3 families, number of smutted plants in various percentage classes among the respective F_4 families, and the parents grown among the latter, of an oat cross, Gopher \times Black Mesdag, and the reciprocal, grown in 1928.

Parent or progeny	F ₃ families		Number of plants in various smut-percentage classes among F ₄ families																	Total
	Plants	Smutted	0	2.5	7.5	12.5	17.5	22.5	27.5	32.5	37.5	42.5	47.5	52.5	57.5	62.5	67.5			
	Number	Per cent																		
Gopher				3	3	3												9		
Black Mesdag			9															9		
16-15-18	63	20.6	2	1	1	1												5		
16-15-3	60	0	5															5		
16-15-51	65	30.8							1			2			1	1		5		
16-15-29	64	0	5															5		
16-15-74	46	33.3						1	1	1					1		1	5		
16-15-41	55	0	5															5		
16-15-77	66	40.9				1					1		1	2				5		
16-15-49	63	0	5															5		
17-10-2	67	37.3			1	1	1	1	1									5		
17-10-8	69	0	5															5		
17-10-10	58	36.2					1			1	2	1						5		
17-10-15	62	0	3	1		1												5		
17-10-17	68	22.1								1	1	2			1			5		
17-10-27	65	0	5															5		
17-10-43	75	42.7						1	1	1	1				1			5		
17-10-47	68	0	5															5		
17-10-60	72	34.7			1			1		2			1					5		
17-10-63	70	38.6					1	1			1			2				5		

Strain 16-15-18 with an infection of 20.6 per cent smut in F_3 produced both smutted and smut-free F_4 families, whereas strain 17-10-17, with approximately the same degree of infection (22.1 per cent) in F_3 , produced nothing but rather highly susceptible F_4 lines. The remaining eight strains, which ranged in smut infection from 30.8 to 42.7 per cent in F_3 , produced only F_4 families which contained some smutted plants.

From the data reported it seems reasonable to conclude that the zero-infection F_3 lines are homozygous, that the low-infection F_3 lines are for the greater part heterozygous, and that the moderately high and the highly susceptible F_3 lines are probably homozygous for at least one main factor for susceptibility.

F_5 FAMILIES

The F_5 families and the parents grown among them in 1928, together with the ancestral F_4 lines grown in 1927, are shown in Table 4. In general, the F_5 lines derived from zero-infection F_4 families bred true to this condition. The exceptions were strains 16-15-31-1 and 16-15-53-3, each of which produced one F_5 family that contained a single smutted plant.

Family 16-15-8-5 contained 10.2 per cent smutted plants in 1927. From Table 2 it may be seen that three of the sibs of this family were recorded as zero infection, and one fell in the class of 2.5 per cent infection. The five F_5 families from line 16-15-8-5 (Table 4) fell into the following classes of percentage infection: One, zero; three, 2.5; and one, 12.5.

Family 16-15-7-5 was one of four sibs which showed a low percentage of smut infection in 1927. The other related F_4 line showed a zero percentage infection. In F_5 , of the five descendant lines from 16-15-7-5, but one showed any smut and that one in a single plant only. Some of these lines may have been susceptible but escaped infection or, if infected, failed to give external evidence of it. A further test of these strains will be made in the F_6 generation.

TABLE 4.—Percentage of smutted plants among the F_4 families, number of smutted plants in various percentage classes among the respective F_5 families, and the parents grown among the latter, of an oat cross, *Gopher* × *Black Mesdag*, and the reciprocal

Parent or progeny	F_4 families		Number of plants in various smut-percentage classes among F_5 families							Total
	Plants	Smutted	0	2.5	7.5	12.5	17.5	22.5	27.5	
	Number	Per cent								
Gopher			2							14
Black Mesdag P.			14	4	5	3				14
16-15-4-1	62	0	1							1
16-15-4-2	64	0	1							1
16-15-4-4	55	0	1							1
16-15-4-5	59	0	1							1
16-15-8-1	45	0	5							5
16-15-8-5	49	10.2	1	3		1				5
16-15-7-3	33	0	5							5
16-15-7-5	59	1.7	4	1						5
16-15-16-2	70	0	2							2
16-15-16-4	67	0	3							3
16-15-31-1	64	0	4	1						5
16-15-31-5	61	1.6	2	2	1					5
16-15-1-5	55	0	5							5
16-15-35-1	54	0	3							3
16-15-17-2	63	0	5							5
16-15-53-3	69	0	2	1						3
16-15-35-4	59	0	3							3
16-15-68-5	51	2.0		2	2	1				5
17-10-1-1	53	0	3							3
17-10-62-1	50	0	3							3
17-10-48-1	51	0	3							3
17-10-1-2	64	0	3							3
17-10-30-1	43	2.3	5							5
17-10-53-2	45	0	3							3
17-10-48-2	55	0	3							3
17-10-1-3	64	0	3							3
17-10-62-3	55	0	3							3
17-10-48-3	50	0	3							3
17-10-1-4	65	0	3							3
17-10-53-3	58	0	3							3
17-10-1-5	61	0	3							3
17-10-53-5	60	0	3							3
17-10-48-5	57	0	3							3
17-10-48-6	46	0	2							2
17-10-48-7	38	0	2							2
17-10-48-8	54	0	2							2
17-10-48-9	54	0	2							2
17-10-48-10	55	0	2							2
16-15-17-6	51	0	4							4
17-10-68-1	46	34.8		1	1				1	3
17-10-68-5	57	38.6			1	1		1		3
17-10-64-1	50	10.0	2		1					3
17-10-64-2	57	30.0		2			1			3

Line 16-15-31-5 is one of three F_4 families with the same ancestry that showed a low percentage of smut. The other two related F_4 families showed no smut. The F_5 descendants of 16-15-31-5 showed percentages of smut as follows: Two families with zero infection; two families which fell into the 2.5 per cent class; and one family into the 7.5 per cent class. In other words, these five F_5 families which descended from 16-15-31-5 reacted to smut in a manner very similar to that of the five F_4 families from 16-15-31.

Strain 16-15-68-5 was one of five F_4 lines with the same ancestry which showed a low percentage of smut infection. It is obvious from Table 4 that the five F_5 lines descended from this strain also showed a low percentage of smut infection, indicating homozygosity for the character.

In 1927 strain 17-10-30-1 was the only one of five F_4 families from the same F_3 line which showed any smut, and this strain showed smut in but a single plant. Although precaution was taken to prevent an accidental mixture, there is always the possibility of contamination from this source. Then, too, there is an appreciable amount of natural crossing in oats at Morgantown, as has been shown in a previous publication.⁶ Whatever the cause of the single apparently susceptible plant observed in the F_4 family, none of the F_5 descendants showed any smut. The evidence strongly indicates that the F_3 line 17-10-30 was homozygous for the main factor controlling smut reaction.

Three F_5 families were grown from low-infection strain 17-10-64-1. Two of these showed no evidence of smut and one showed a percentage of smut that fell into the 7.5 class. This result was hardly expected, as F_4 strain 17-10-64-1 was one of five which showed smut. A further test in the F_6 generation will be made. This is of particular importance because of the fact that two of the duplicate plots of Gopher did not show any evidence of smut infection in 1928.

The three remaining F_4 strains which showed smut in 1927 and which were tested in F_5 were classed as moderately high in susceptibility. Each one of the three produced one F_5 family which showed a degree of smut infection greater than the most susceptible duplicate plots of Gopher.

PEDIGREES OF CERTAIN F_5 LINES

In order to bring some of the pedigrees together for comparison Table 5 has been prepared. It will be observed that all of the lines listed in the table which gave no evidence of smut infection in F_3 bred true for this condition through the F_5 generation. Two of the three F_3 families which showed a relatively low smut infection produced both infected and noninfected descendants. The third family, 16-15-31, produced three infected and two noninfected F_4 lines, and of the five F_5 families grown from one of the noninfected strains four showed no evidence of smut and one showed smut in a single plant only. The data seem to indicate the possibility that 16-15-31 also was heterozygous for smut reaction. Strain 16-15-68 contained 28.8 per cent smutted plants in F_3 , a percentage well within the range exhibited by the Gopher parent grown the same year. The five F_4 families likewise showed percentages of smutted plants similar to Gopher grown that year, and again in F_5 the Gopher parent and the five families descended from one of the F_4 lines showed similar reaction, thus indicating that the genotypes of these lines are similar to that of one of the parents. The highly susceptible F_3 families, 17-10-64 and 17-10-68, produced only susceptible F_4 lines. The group from the former ranged from 10.0 to 29.8 per cent of smutted plants and from the latter 29.8 to 42.9 per cent. However, in F_5 two families descended from 17-10-64 and one from 17-10-68 gave

⁶ GARBER, R. J., and QUISENBERRY, K. S. NATURAL CROSSING IN OATS AT MORGANTOWN, WEST VIRGINIA. *Jour. Amer. Soc. Agron.* 19: 191-197. 1927.

no evidence of infection. This fact may or may not be of genetic significance, particularly in view of the somewhat erratic smut epidemic obtained among the F_3 families. Further tests are being made. With the technic used in this experiment considerable variation in the percentages of smutted plants among the highly susceptible line was expected, but families without any smutted plants were not expected. It is evident from Table 5 that some of the F_3 families from the two highly susceptible F_3 lines again showed transgressive susceptibility.

TABLE 5.—*Pedigrees and number of smutted plants in various percentage classes among certain F_3 lines of the oat cross, Gopher \times Black Mesdag, and the reciprocal*

Progeny	Smutted in F_1	Progeny	Smutted in F_1	Number of plants in various smut-percentage classes among F_3 families							
				0	2.5	7.5	12.5	17.5	22.5	27.5	5
	<i>Per cent</i>		<i>Per cent</i>								
16-15-7	12.5	16-15-7-3	0	5							
		16-15-7-5	1.7	4	1						
16-15-8	1.5	16-15-8-1	0	5							
		16-15-8-5	10.2	1	3		1				
16-15-16	0	16-15-16-2	0	2							
		16-15-16-4	0	3							
16-15-17	0	16-15-17-2	0	5							
		16-15-17-6	0	4							
16-15-31	1.5	16-15-31-1	0	4	1						
		16-15-31-5	1.6	2	2	1					
16-15-35	0	16-15-35-1	0	3							
		16-15-35-4	0	3							
16-15-68	28.8	16-15-68-5	2.0		2	2	1				
		17-10-1-1	0	3							
17-10-1	0	17-10-1-2	0	3							
		17-10-1-3	0	3							
		17-10-1-4	0	3							
		17-10-1-5	0	3							
		17-10-48-1	0	3							
		17-10-48-2	0	3							
		17-10-48-3	0	3							
		17-10-48-5	0	3							
17-10-48	0	17-10-48-6	0	2							
		17-10-48-7	0	2							
		17-10-48-8	0	2							
		18-10-48-9	0	2							
		18-10-48-10	0	2							
17-10-53	0	17-10-53-2	0	3							
		17-10-53-3	0	3							
		17-10-53-5	0	3							
17-10-62	0	17-10-62-1	0	3							
		17-10-62-3	0	3							
17-10-64	50.7	17-10-64-1	10.0	2		1					
		17-10-64-2	30.0		2			1			
17-10-68	65.5	17-10-68-1	34.8	1		1				1	
		17-10-68-5	38.6			1	1		1		

INHERITANCE OF SMUT REACTION

A considerable number of F_1 plants have been grown in connection with this investigation, but even though the crossed seed was treated with smut spores no smutted F_1 plants have been found. In 1928 some crossed seed was hulled, treated with smut, and then planted. Of the 23 plants produced, none showed any evidence of infection. Resistance apparently behaved as a dominant in the cross Gopher \times Black Mesdag and the reciprocal.

In considering the inheritance of smut reaction it may be well first to reexamine the 100 F_3 families grown in 1926, when a severe

epidemic was obtained, and take into account the behavior of the F_4 generation. To aid in this reexamination Table 6 has been prepared. A family was considered as segregating if it produced both infected and noninfected descendant lines. Of course this classification does not take into account families which may be segregating for degree of susceptibility.

TABLE 6.—Breeding behavior of certain F_3 families as revealed in the F_4 generation of an oat cross, *Gopher* × *Black Mesdag*, and the reciprocal

Smut percentage class in F_3	Breeding nature of F_3 plants as shown in F_4	F_3 families
		Number
0	{ Homogynous resistant	19
	{ Segregating	2
0.1- 9.9	{ do	4
10 -19.9	{ do	3
20 -29.9	{ Homogynous susceptible	4
	{ Segregating	3
30	{ Homogynous susceptible	13

It is apparent from Table 6 that of the 21 F_3 families which showed no smut, 19 bred true to this condition in F_4 . In all cases at least 5 F_4 families were grown from each F_3 line that was tested. The 2 F_3 lines which segregated in F_4 should have been classified as low-infection strains. The 7 F_3 families which showed a percentage of smut infection between 0 and 19.9 segregated in the next generation, and of the 7 F_3 lines with a percentage of smutted plants from 20 to 29.9, inclusive, 4 bred true and 3 segregated. All of the 13 F_3 lines with a percentage of smutted plants 30 or above bred true. In view of this situation, the 100 F_3 families grown in 1926 (Table 1) may be classified as follows: Nineteen bred true for zero infection; 64 segregated and 17 bred true for more or less infection, a ratio which departs rather significantly from monohybrid expectation ($P \approx$ approximately 0.03 with 2 degrees of freedom⁷). If the segregating families and those which bred true for more or less infection are grouped together, the deviation (6.0 ± 2.9) is hardly significant.

It will be observed from Table 6 that only seven F_3 families which showed percentages of smutted plants between 0.1 and 19.9 were tested in F_4 . It is possible that if more families in this F_3 group had been grown, a few homozygous ones would have been found. Moreover, it should be pointed out that a few F_2 plants, from which the F_3 lines were derived, were eliminated because they were infected with smut and produced little if any seed. One or both of these causes may have been instrumental in producing the departure from expectation noted above.

Considering the 50 F_3 families grown in triplicate plots in 1928 (Table 1) and placing them in two classes, those which showed no smutted plants and those which did, a ratio of 13 to 37, respectively, is obtained; a very close agreement with a 1:3 ratio. It seems reasonable to conclude that one main factor difference was operative in determining the resistance or immunity of the *Black Mesdag* parent in this cross.

⁷ FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. 239 p., illus. London, 1925.

The data collected from the F_3 generation for the most part bore out the conclusion just stated. Some irregularities did appear, but they may have been due to nongenetic causes. Further tests are being made in the F_6 generation.

TRANSGRESSIVE SEGREGATION

Families were obtained in the F_3 and later generations which contained a distinctly greater percentage of smutted plants than did the susceptible parent grown in close proximity, thus indicating that transgressive segregation had taken place. It seems probable that the Black Mesdag parent brought into the cross one or more factors which increased susceptibility when associated with the susceptibility factor of Gopher. The results obtained thus far may be explained fairly satisfactorily by assuming that Black Mesdag carries a dominant factor (R) which gives high resistance, if not actual immunity, and another factor (i) which when associated with the homozygous condition for susceptibility (r) of the Gopher parent results in transgressive segregation. The reaction of Gopher to smut suggests that the I factor carried by it may act as an inhibitor to r or that it is an additional factor for resistance but less potent than the R factor carried by Black Mesdag.

A tentative factorial analysis follows:

Parents:	F_2	F_3
Black Mesdag, $R R i i$.	$R R I I$	
Gopher, $r r I I$.	$r r i i$	
	$2 R R I i$	Breed true for resistance.
	$R R i i$	
	$2 R r I I$	
	$4 R r I i$	Low susceptibility and segre-
	$2 R r i i$	gate in F_4 .
	$r r I I$	Breed true for susceptibility
		of Gopher.
	$2 r r I i$	Somewhat more susceptible
		than Gopher.
	$r r i i$	Breed true for high suscep-
		tibility.

On the basis of this hypothesis approximately one-sixteenth, or 9.4, of the 150 F_3 families would be expected to show transgressive susceptibility. There were 13 such families actually obtained, i. e., families which showed percentages of smutted plants greater than the most susceptible plots of the Gopher parent. The departure from expectation is 3.6 ± 2 .

The F_3 families homozygous for R (should) breed true for zero infection, and for the most part such results were actually obtained. The F_3 families from F_2 plants heterozygous for R (should) show low susceptibility similar to Gopher, but (should) show segregation in the F_4 generation. The seven F_3 families which showed a low infection and the two additional F_3 families which did not show any smutted plants, but probably should have been classified as low-infection strains, all segregated in F_4 . Of the seven F_3 families which showed percentages of smutted plants from 20 to 29.9, inclusive, four segregated and three showed some smutted plants, but only one (16-15-68) of this latter group apparently bred true for the degree of susceptibility of the Gopher parent. On the basis of the above hypothesis about one-ninth of the slightly susceptible F_3 strains would be expected to breed true.

Mention already has been made of the fact that the black-color gene *B* of the Mesdag parent seems to be linked with the *i* factor. Certain back crosses have been made, and it is hoped that the data obtained from these will not only clear up this linkage relation, but will also clarify the inheritance situation.

SUMMARY AND CONCLUSIONS

Gopher, a pure-line selection of oats moderately susceptible to smut, was crossed with a highly resistant if not immune pure-line selection of Black Mesdag. The inheritance of resistance to smut in relation to seed color has been studied through the F_5 generation.

Resistance to smut is apparently dominant and controlled by a single main factor difference. Evidence of a modifying factor or group of factors was also obtained. Certain lines descendant from the cross showed distinctly greater susceptibility to smut than did the Gopher parent grown at the same time.

There is apparently a linkage between the black-color gene and a modifying factor which is bringing about transgressive segregation for susceptibility.

THE VITAMIN-C CONTENT OF FRESH SAUERKRAUT AND SAUERKRAUT JUICE¹

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INTRODUCTION

There is a considerable amount of advertising before the public proclaiming the value of sauerkraut and sauerkraut juice as sources of the vitamins. Statements such as "contains all the vitamins" and "rich in vitamins A, B, C, and D" have been used, but these statements appear to be based upon general observations rather than upon experimental evidence. In some cases it is said that cabbage contains all the vitamins, and the inference is drawn that sauerkraut likewise contains all the vitamins. In view of the well-known instability of vitamin C, it appears legitimate to raise the question whether this vitamin is present in a product which has undergone such operations as are involved in the making and marketing of sauerkraut.

Some justification for the belief that sauerkraut must contain vitamin C is found in old reports regarding its use as an antiscorbutic agent on long sea voyages and the freedom from scurvy in certain regions where sauerkraut was extensively used as a food (4, p. 45).² But many other and often unobserved factors operate in such cases, and it is unsafe to place much reliance on these old reports.

A review of the literature seems to show only two pieces of experimental work on the vitamin-C content of sauerkraut. Ellis, Steenbock, and Hart (3), in their study of the stability of the antiscorbutic vitamin and its response to various treatments, include data on raw sauerkraut obtained from a local grocery. In this work they fed the sauerkraut at 2.5 and 5 gm. levels (daily) to guinea pigs on a scorbutic ration. On the 2.5-gm. level the animals died of scurvy within four to five weeks, while those on the 5-gm. level died in about six weeks. The conclusion of Ellis and his associates was that at these levels the sauerkraut showed no evidence of possessing antiscorbutic properties.

In a later paper, Wedgewood and Ford (9) report that sauerkraut juice in quantities ranging from 0.5 gm. to 5 c. c. per day did not prevent scurvy in guinea pigs. They are not very explicit as to how the sauerkraut was made, and it is therefore difficult to decide whether or not their sauerkraut can be taken as representative of the commercial product. Their work is also open to the criticism that the sauerkraut juice used may have lost its potency as a result of storage. The juice for the entire feeding period (26 to 33 days) was expressed from the sauerkraut at the beginning of the experiment and kept on

¹ Received for publication Apr. 13, 1929; issued December, 1929. Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

² Reference is made by number (italic) to "Literature cited," p. 971.

ice until it was fed. Since the juice was not kept under anaerobic conditions, it is probable that, even if present at the beginning of the feeding period, vitamin C would have disappeared from the juice in the course of the experiment.

In view of the lack of experimental evidence available, it seemed desirable to make a study of the vitamin-C content of sauerkraut.

EXPERIMENTAL WORK

Two rations were used in this work. The first is a modification of that employed by Cohen and Mendel (1) and also by Parsons and Reynolds (6). The ration as given below has been used in this laboratory for the past three years and is called the soybean scurvy ration. Its composition is as follows: Soybean flour, 1,620 gm.; dried yeast, 120 gm.; purified casein, 105 gm.; calcium carbonate, 60 gm.; filter paper, 40 gm.; butterfat, 100 gm. The soybean flour was a commercial product the preparation of which involves heating. The yeast also was a commercial product and was a reliable source of the vitamin-B complex. The casein was purified by soaking for a week in water slightly acidified with glacial acetic acid (about 5 c. c. per 6 quarts of tap water). This water was changed every day. The filter paper was cut into small pieces and beaten in distilled water until a fine pulp was obtained. This was poured upon the mixed dry ingredients and the mass was rubbed until evenly mixed. When dry, it was ground and mixed thoroughly with melted butterfat.

The second ration is that used by Ellis, Steenbock, and Hart (3). It is called the alfalfa scurvy ration and contains the following: Alfalfa (autoclaved 30 minutes at 15 pounds pressure), 25 per cent; rolled oats, 69 per cent; purified casein, 5 per cent.

Animals that were used as positive or negative controls were given the above rations with the addition of 60 gm. of sodium chloride to the soybean ration and 1 per cent sodium chloride to the alfalfa ration. Animals fed sauerkraut or sauerkraut juice received their sodium chloride from these materials.

In the early part of the work both rations were used, but since the two rations were found to be quite similar from the standpoint of production of scurvy, only the alfalfa ration was used in the later experiments.

The protection of guinea pigs from scurvy was the method used in the investigation. After the preliminary feeding period described below, cabbage was removed from the diet and sauerkraut only was fed with the basal ration for a period of 60 to 75 days or until the animal died. The onset of scurvy symptoms, swollen wrists or a "jerky run," was noted and autopsy performed at the end of the experiment or after death.

On autopsy, the following signs of scurvy were looked for: Hemorrhages and swelling of the wrists or elbows of the fore legs and also below the hip joint of the hind legs; hemorrhages or bleeding at the costochondral juncture of the ribs, loose teeth, and any possible hemorrhages in the abdominal organs or in the peritoneum. The condition of the residue in the intestinal tract was also noted.

The weight of the guinea pigs when the cabbage was removed from the diet and sauerkraut feeding was begun varied from 211 to 276 gm., the average being about 240 gm. Young guinea pigs when received in

the laboratory or when taken from the mother were placed on the scurvy ration plus cabbage and allowed to eat *ad libitum*. With the first group of guinea pigs there was no preliminary feeding of sauerkraut before the experiment was begun, and difficulty was experienced in getting some of the animals to eat their portions. With all the other groups the following procedure was used: On the first day the basal ration plus cabbage only was fed; on the second day the cabbage was ground in a food chopper, a small amount (5 to 10 gm.) of sauerkraut was added to the cabbage, and the mixture was put into the basal ration. The sauerkraut was increased each day, but at no time was the amount of cabbage in the mixture less than 5 gm. per guinea pig in the group. In this way the guinea pigs gradually became accustomed to the taste of the sauerkraut, and as a general rule there was no trouble in getting them to eat it. When the experiment was begun each animal was placed in a separate cage, and the dose of fresh sauerkraut was mixed with the ration. The fresh sauerkraut juice was given to the animals by means of a medicine dripper.

The sauerkraut used in this experiment was made from All Seasons variety of cabbage, cut during the first week in November, 1927, and stored outside in a cool place for a week before it was made into sauerkraut. Three hundred pounds of shredded cabbage were mixed with $7\frac{1}{2}$ pounds of salt and packed into 45-gallon barrels. A loose-fitting cover held down by a heavy weight was placed on top of the cabbage. These barrels of cabbage were allowed to ferment for 90 days at a temperature which ranged from 60° to 65° F. After this time the barrels were opened and the top sauerkraut, to the depth of 1 foot, was discarded. The sauerkraut was of good quality and possessed an acidity of 1.7 per cent calculated as lactic acid. Each day fresh samples were removed from points well below the surface, thus reducing or eliminating the factor of oxidation. The sauerkraut was ground in a food chopper and fed to the animals within as short a time as possible. The sauerkraut juice was obtained by putting the sauerkraut in a fine-meshed canvas bag and squeezing out the juice.

The study was made on three levels: Group 1 on 10 gm. of sauerkraut or sauerkraut juice daily; Group 2 on 5 gm. of sauerkraut daily; and Group 3 on 2.5 gm. of sauerkraut daily.

In the first group, three guinea pigs that failed to eat their portions were given partly neutralized sauerkraut in order to stimulate consumption. After the acidity of the sauerkraut had been determined, each day's sample was half neutralized with N/1 NaOH immediately before feeding. These animals (Nos. 4, 5, and 15) seemed to eat the partly neutralized sauerkraut better than the unneutralized. They were fed this type of sauerkraut throughout the 60 days of the experiment.

DISCUSSION OF RESULTS

HIGH LEVEL OF SAUERKRAUT AND SAUERKRAUT JUICE (10 gm.)

The first group of guinea pigs were fed 10 gm. daily of sauerkraut or sauerkraut juice. Inasmuch as Ellis, Steenbock, and Hart (3) reported no antiscorbutic value at 2.5 and 5 gm. levels, it seemed advisable to begin with the higher level in order to insure the detection of any vitamin C if it were present.

The individual weight curves (figs. 1 and 2) show that a 10-gm. level of this sauerkraut or the juice therefrom gave good growth and

complete protection from scurvy. The daily gain in weight of the 7 animals which received juice ranged from 2.26 to 5.33 gm. and averaged 3.83 gm. The figures for the 8 animals fed sauerkraut

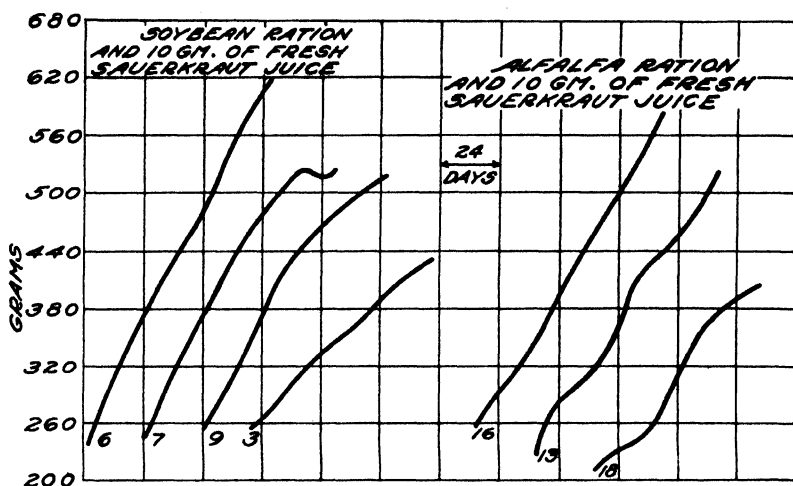


FIGURE 1.—Growth curves showing the antiscorbutic effect of 10 gm. of fresh sauerkraut juice daily when added to two scurvy rations fed to guinea pigs; autopsies at the end of 75 days disclosed no evidences of scurvy in any of the animals

ranged from 2.22 to 4.85 gm. and averaged 3.53 gm. The 2 animals which received 5 gm. of raw cabbage daily (fig. 4) made daily gains of 4.06 and 4.90 gm., respectively, and averaged 4.48 gm. Of the 15 animals fed sauerkraut or its juice at this level 6 grew faster than 1 of the animals (No. 11) that received 5 gm. of cabbage, and 3 made greater

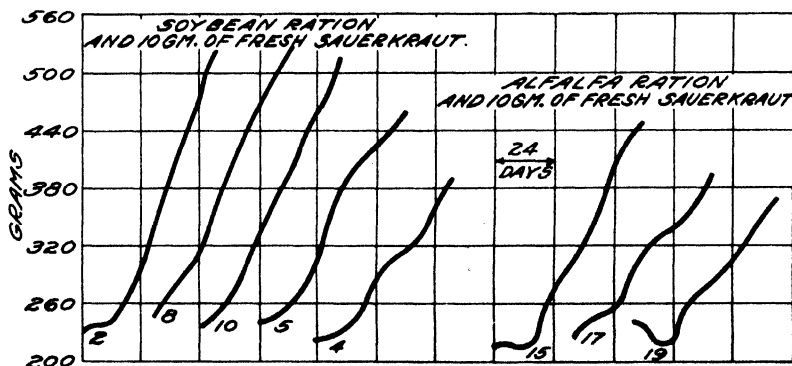


FIGURE 2.—Growth curves showing the antiscorbutic effect of 10 gm. of fresh sauerkraut daily when added to two scurvy rations fed to guinea pigs; autopsies at the end of 60 days disclosed no evidences of scurvy in any of the animals

daily gains than either of the positive controls. Because of the limited number of animals used as cabbage controls, too much emphasis should not be placed on the comparison, but it appears that 10 gm. of sauerkraut or sauerkraut juice contain about the same amount of vitamin C as 5 gm. of raw stored cabbage.

MEDIUM AND LOW LEVELS OF SAUERKRAUT (5 and 2.5 gm.)

When, after six to seven weeks, it was evident that the 10-gm. levels of sauerkraut and sauerkraut juice were each giving protection from scurvy and allowing good growth, feedings at 5 and 2.5 gm. levels

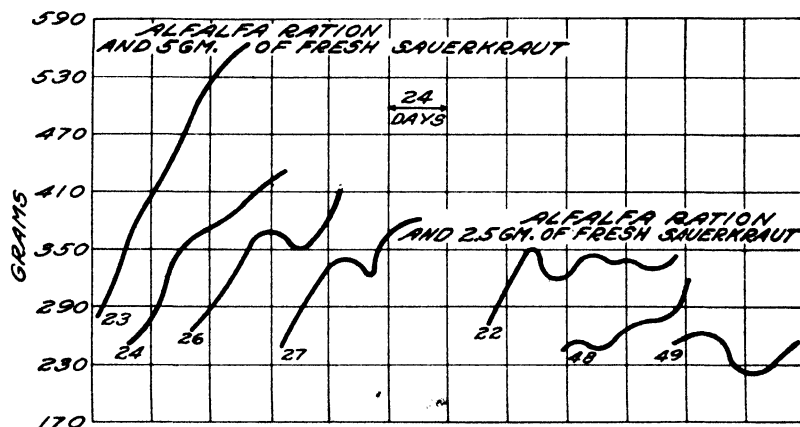


FIGURE 3.—Growth curves showing the antiscorbutic effect of 5 and 2.5 gm. of fresh sauerkraut daily when added to the alfalfa scurvy ration fed to guinea pigs. Autopsies performed at the end of 60 days on the animals fed at the 5-gm. level disclosed no evidences of scurvy in any case. An autopsy on animal No. 22 at the end of 75 days showed brittle leg bones; that on No. 48 at the end of 55 days, some swelling and muscular hemorrhage; that on No. 49, a slight swelling on one leg. These last three animals received but 2.5 gm. of fresh sauerkraut daily.

were begun. Although growth on a 5-gm. level was not so consistently good as on the 10-gm. level, there was no evidence of scurvy. The daily gain in weight of the four guinea pigs receiving 5 gm. of

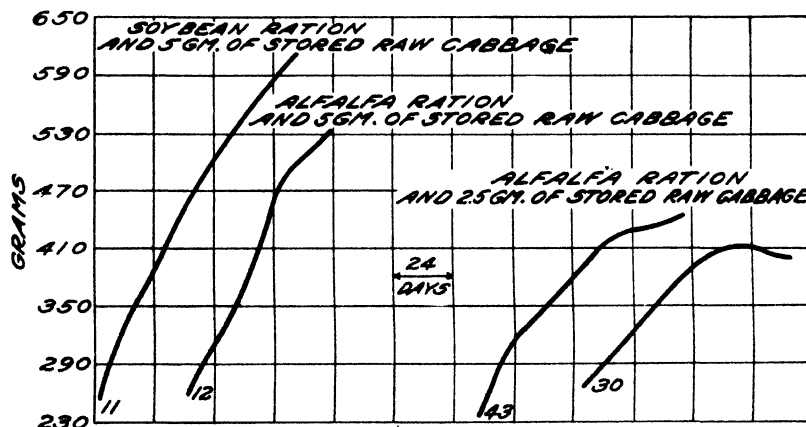


FIGURE 4.—Growth curves showing the antiscorbutic effect of 5 and 2.5 gm. of stored raw cabbage daily when added to two scurvy rations fed to guinea pigs.

sauerkraut ranged from 2.33 to 4.97 gm. and averaged 3.30 gm. (Fig. 3.) This average gain is slightly lower than that for the animals on 10 gm. of juice or sauerkraut, but two of the guinea pigs, Nos. 23 and 24, have weight curves which are similar to many of the weight

curves of animals receiving 10 gm. of sauerkraut. It seems evident that a 5-gm. level of this sauerkraut was well within the protection level and also allowed good growth.

The growth on the 5-gm. level of sauerkraut was better than that obtained from one-half this quantity of cabbage. The gain per day on 2.5 gm. of cabbage (Fig. 4) was 2.92 gm. as compared with 3.30 on 5 gm. of sauerkraut. As already pointed out in the comparison of the higher levels of sauerkraut and of cabbage, the quantity of vitamin C in the sauerkraut was approximately equal to one-half of that contained in the cabbage.

Six animals were started on a 2.5-gm. level of sauerkraut, but only three of them lived through the experimental period. Three guinea pigs grew fairly well for 16 to 36 days, but at the end of this time there was a sudden drop in weight which was accompanied by a refusal to eat. The autopsy showed abnormal intestinal conditions. Guinea pig No. 22 was chloroformed at the end of 75 days instead of the usual 60 because of the several periods of loss and gain in weight of this animal. Although there were numerous fluctuations in the weight curve, it is evident that from the twentieth to the seventy-fifth day the animal was merely maintaining its weight. On autopsy, the leg bones were found to be very brittle and were easily fractured. Although there were no signs of hemorrhage, the brittle leg bones may have been due to a latent condition of scurvy.

Guinea pigs Nos. 48 and 49, also receiving 2.5 gm. of sauerkraut but started a month later than any of the other animals, were chloroformed at the end of 55 days because the sauerkraut was so nearly gone that satisfactory samples below the surface could not be obtained. Again, the weight curves (fig. 3) seem to indicate that 2.5 gm. of sauerkraut are needed for a maintenance level. Autopsy showed that with both animals there was slight evidence of scurvy.

Eddy et al. (2) consider 1 gm. of raw cabbage as the minimum protective dose. Unpublished data from this laboratory obtained by DeVilbiss showed that 1 gm. of raw cabbage added daily to Sherman's (8) basal diet would support life for 90 days, but the autopsy revealed brittle bones and teeth. In the present sauerkraut study 2.5 gm. of raw cabbage was the lowest level used. This level gave good growth for 44 to 58 days, and after that time the weight curve flattened off. (Fig. 4.) If 1 gm. of raw cabbage is considered to be the minimum protective level, with perhaps slight signs of scurvy, and 2.5 gm. of fresh sauerkraut the minimum level although not completely protective, it appears that approximately one-half of the vitamin-C content of cabbage may be destroyed in the process of making sauerkraut.

The experiments of Ellis, Hart, and Steenbock (3) showed a much greater destruction of vitamin C than is indicated in the present studies. These authors, however, used bulk sauerkraut obtained from a grocery store. It is probable that much of the vitamin-C content was lost after the sauerkraut was taken from the fermentation vat and repacked. As it frequently takes several weeks for a grocer to market a barrel of sauerkraut, it is not improbable that the vitamin-C content of the sauerkraut was lost by exposure to the air during the period of retailing.

That oxidation and not fermentation is the important factor in the destruction of vitamin C was shown later in the same laboratory

by Lepkovsky, Hart, Hastings, and Frazier (5). Sterile orange juice and tomato juice kept in test tubes plugged with cotton showed marked deterioration in 7 and 24 days, respectively. If air was excluded by means of vaseline plugs the juices retained their potency. Likewise, if the tomato juice was sealed with vaseline plugs and fermented with bacteria, some of which are characteristic of sauerkraut, the juice was still effective in the cure of scurvy. An earlier paper by Zilva (10) showed that the sugars present in lemon juice could be removed by yeast without appreciably altering the vitamin-C content of the juice.

It is not improbable that the strictly fermentation processes which take place in the formation of sauerkraut tend to prevent the destruction of vitamin C. In a recent paper Pruess, Peterson, and Fred (7) report that the gases formed in the fermentation are nearly 100 per cent carbon dioxide. The oxygen incorporated in the shredded cabbage at the time it is placed in the vat is probably speedily consumed by the respiration of the plant cells or removed from the mass by the carbon dioxide formed by the plant cells and bacteria. However, a trace of oxygen seems to persist in the sauerkraut throughout the fermentation and the partial destruction of vitamin C is probably due to this rather than to the specific action of the microorganisms.

The authors wish to point out specifically that the presence of vitamin C in the sauerkraut used in the present study does not warrant the assumption that sauerkraut as it reaches the consumer contains vitamin C. Indeed, the experiments of Ellis, Hart, and Steenbock (3) show that in some instances it does not. Whether or not canned sauerkraut or commercial sauerkraut juice contains vitamin C can be decided only by carefully controlled experiments that deal directly with these products.

PRESENCE OF VITAMIN C DEMONSTRATED BY RECOVERY TYPE OF EXPERIMENT

Two guinea pigs in the "negative" group, Nos. 32 and 67, were given 5 c. c. of sauerkraut juice at the onset of definite scurvy symptoms. (Fig. 5.) The juice was fed for 16 days and autopsies were performed on the animals on the seventeenth day. The autopsies indicated in both cases that the animals were practically cured of scurvy. Although the protection method was used primarily in this study, the above-mentioned data on the recovery of two of the "negative controls" strengthen the conclusion that an appreciable quantity of vitamin C was present in this sauerkraut juice.

SUMMARY

This study shows the vitamin C content of fresh raw sauerkraut and sauerkraut juice, i. e., material which was fed immediately after being taken from the barrel in which it was fermented. The sauerkraut was fed at levels of 10, 5, and 2.5 gm. daily for each guinea pig for approximately 60 days, and sauerkraut juice was fed at a level of 10 gm. for 75 days. Positive control animals were fed at 5 and 2.5 gm. levels of raw stored cabbage.

The 10-gm. level of either fresh sauerkraut or fresh juice protected from scurvy and allowed good and in some cases excellent growth. The growth curves are comparable to those of guinea pigs receiving

5 gm. of raw stored cabbage. The 5-gm. dosage of fresh sauerkraut was well within the protection level and also allowed good growth. The 2.5 gm. level of fresh sauerkraut was probably not quite enough to protect from scurvy, although a maintenance of weight was obtained. The 2.5 gm. level of raw stored cabbage allowed good growth for 44 to 58 days, and after that time the weight curve

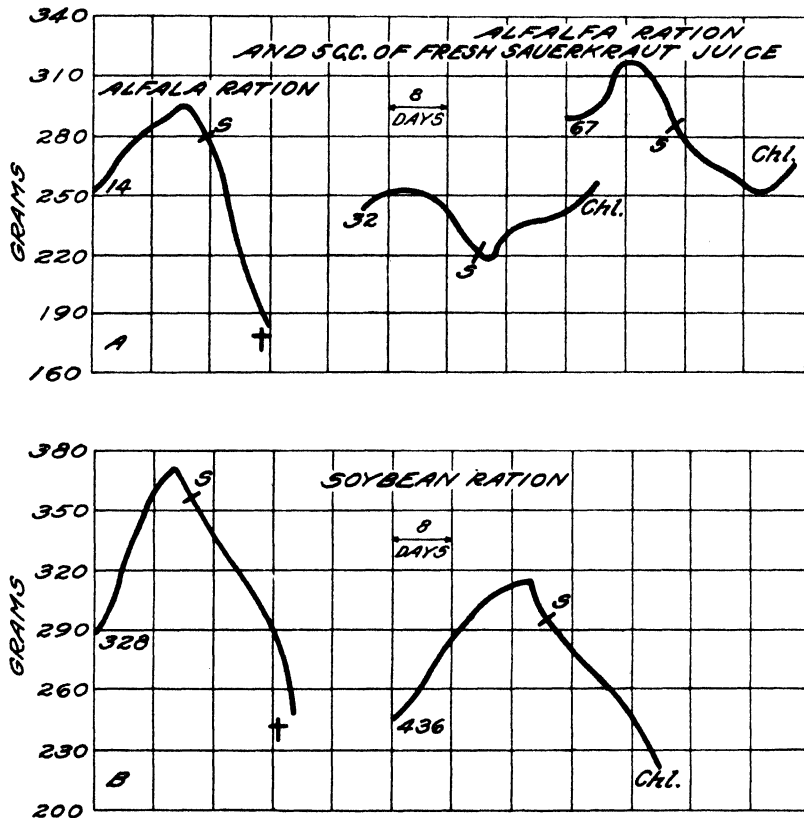


FIGURE 5.—Growth curves of guinea pigs receiving only the basal scurvy-producing rations. Definite symptoms of scurvy were present at points marked "S"; + indicates death and "chl." indicates that the animal was chloroformed. A. These animals were on the alfalfa scurvy ration. Nos. 32 and 67 were given a recovery dosage of 5 c. c. of fresh sauerkraut juice daily; autopsy at the end of a recovery period of 16 days disclosed that recovery was complete. Animal No. 14 was a "negative control" receiving no addition to the basal diet and showed, on autopsy, characteristic evidences of scurvy. B. These animals were on the soybean scurvy ration and, at autopsy, showed the characteristic evidences of scurvy.

"flattened off," although there was no evidence of scurvy. A loss of vitamin C in the formation of sauerkraut is thus definitely demonstrated. This loss is approximately one-half of the vitamin-C content of cabbage.

Guinea pigs which had developed definite symptoms of scurvy recovered when fed 5 c. c. of fresh sauerkraut juice daily.

No conclusions as to the probable vitamin-C content of commercial sauerkraut are warranted by these experiments.

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